INFLUENCE OF ELECTRO-ACUPUNCTURE ON PAIN THRESHOLD AND LAMENESS IN HORSES AND ITS MODE OF ACTION

BY

HUISHENG XIE

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This dissertation is dedicated to my wife Yanru Zhao, my daughter Sherrie and my son Tim. They have always provided love and support.

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KEY TO ABBREVIATIONS

ACUI:	the first acupuncture treatment, using acupoints GV-20a, GV-6, SI-9 and SYL at 80-120 Hz in this study
ACUII:	the second acupuncture treatment using acupoints SI-9, SYL, QCW, and QJ at 80-120Hz in this study
ACUIII:	the third acupuncture treatment, using acupoints SI-9, SYL, QCW, and QJ at 20Hz
ACUIV:	the fourth acupuncture treatment, using acupoints either SI-9 and SYL or QCW and QJ at 80-120 Hz
Acupoint:	acupuncture point
Acupoints:	acupuncture points
Arc:	arcuate nucleus
Amy:	nucleus amygdala
BAU:	Beijing Agricultural University
BHSL:	back half stride length
CM:	centrum medianum
CN:	caudate nucleus
C-PMNs:	C-polymodal nociceptors
CSF:	cerebrospinal fluid
DBB:	= FHSL - BHSL
DLF:	dorsolateral funiculi
DPA:	D-phenylalanine
EA:	electro-acupuncture
EAA:	electro-acupuncture analgesia
FHSL:	front half stride length
GB-34:	acupuncture point name, called "Yang-ling-quan"
GV-6:	acupuncture point name, called "Duan-xue" in horses, and "Xuan-shu" in people
GV-20a:	acupuncture point name, called "Bai-hui" in horses
HN:	habenular nucleus
HP:	hippocampus
Hz:	Hertz, electrical frequency
HWRL:	hoof withdrawal reflex latency
LPN:	lateral palmer nerve
MAP:	mean arterial blood pressure
NA:	nucleus accumbens

NCG:	negative control group, 2 ml of saline injected near the lateral palmer nerve
NRM:	nucleus raphe magnus
NTS:	nucleus tractus solitarii
OC:	occipital cortex
OFQ:	orphnin Q
PA:	preoptic area
PAG:	periaquenductal gray
PCG:	positive control group, 2 ml of 0.5% bupivacaine injected near the lateral palm nerve
PT:	pain threshold
QJ:	acupuncture point, called "Qian-jiu"
QCW:	acupuncture point, called "Qian-chan-wan"
RM:	nuclei raphe magnus
RNA:	renal nerve activity
SA:	septal area
SCASVM:	Sichuan College of Animal Science and Veterinary Medicine
SI-9:	acupuncture point, called "Qiang-feng" in horses
SP:	substance P
St-36:	acupuncture point name, called "Zu-san-li" in people or "Hou-san-li" in horses
STT:	spinothalamic tract
SYL:	acupuncture point name, called "San-yang-luo"
TNSA:	transcutaneous nerve stimulation analgesia
TSL:	total stride length
5-HT:	serotonin

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INFLUENCE OF ELECTRO-ACUPUNCTURE ON PAIN THRESHOLD
AND LAMENESS IN HORSES AND ITS MODE OF ACTION

By

Huisheng Xie

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Chairman: E.A. Ott, PhD
Major Department: Animal Science

The purpose of this study was to evaluate the effectiveness of electro-acupuncture (EA) on pain perception and experimental lameness in horses and measure its neuroendocrine responses. In experiment one, 22 Thoroughbred horses were assigned to 6 groups: ACUI - EA at 80-120 Hz, ACUII - EA at 80-120Hz, ACUIII - EA at 20Hz, ACUIV - EA at 80-120 Hz, PCG - 2 ml of 0.5% bupivacaine near the lateral palmer nerve (LPN), NCG - 2 ml saline near LPN. Focused radiant light/heat was used as a noxious stimulus and was directed onto the pastern to elicit the classic flexion-withdrawal reflex. Hoof withdrawal reflex latency (HWRL) is defined as the time (seconds) between lamp illumination and the withdrawal of the hoof. ACUII and ACUIV increased ($P<0.01$) HWRL, while ACUIII did not alter HWRL. The results suggest that EA

with high frequency induces a stronger analgesic effect than that with low frequency. ACUII caused a stronger and longer analgesia than ACUIV, suggesting that acupuncture points potentiate each other.

In experiment two, 6 Thoroughbreds were assigned to 3 treatments: EA, bupivacaine and saline. This was a double 3X3 Latin square design. Lameness was produced in each subject by tightening a set screw against the sole of the hoof. Lameness grading scores of 0,1,2,3 were used to evaluate the severity of lameness. Three types of stride length were measured: total stride length (TSL), front half stride length (FHSL) and back half stride length (BHSL). The difference between FHSL and BHSL was defined as DFB. The DFB increased significantly when the horse was lame, suggesting that the DFB could be used as an objective parameter to measure lameness in horses. EA reduced the lameness score significantly.

Plasma concentrations of β-endorphin, ACTH and cortisol were measured in both experiments. EA significantly increased HWRL and reduced the lameness score, and simultaneously increased the plasma β-endorphin concentration. These results indicate that the release of β-endorphin may be the pathway in which acupuncture relieves the experimental pain. None of the acupuncture treatments altered the ACTH concentrations, which indicates that ACTH is not involved in electro-acupuncture analgesia.

CHAPTER 1
INTRODUCTION

The horse industry is one of the largest animal industries in the USA. From the economics perspective, racing and other performance events are dominant parts of the industry. In the racing community, performance means competition at high speed. As a result, the equine athlete often suffers from fatigue and pain. The recognition and alleviation of pain in horses is a growing veterinary and public concern.

Acupuncture is best known for its effect on pain relief in both horses and people (Fleming, 1994; Richardson and Vincent, 1986). Acupuncture may be defined as the stimulation of a specific point on the body, known as an acupuncture point, or acupoint, with a specific method, resulting in a therapeutic or hemostatic effect. The origin of this therapy can be traced to China as early as 2,200 to 3,000 B.C. (Yu, 1995). Historically, veterinary practitioners have used conventional needles, bleeding needles, heat, moxa, cauterization and massage to stimulate acupoints (Xie, 1994). Modern technology, such as laser, electricity, injectable agents and microwaves, can also be used to stimulate acupoints. Electro-acupuncture (EA) is a method in which an electric current is passed to the animal through a needle inserted an acupoint. EA is generally

considered to have stronger effects than other types of acupuncture methods (Yu, 1984; Panzer, 1992; Liang, 1982). EA has been reported to be effective for treatment of various musculosketetal pain-producing conditions including cervical, thoracolumbar and lumbosacral hyperpathia (Fleming, 1994 and Li, 1993), chronic lameness (Steiss et al., 1989), facial and radial paralysis (Wang and Hu, 1983), dislocation of joints (Chen et al., 1987), degenerative joint diseases (Bao, 1982), back pain (Harman, 1992; Xie et al., 1996 and 1997), colic (Zhou, 1992; Zhu, 1979; and Hu, 1984), infertility (Xie et al., 1994), infection after surgery (Tao, 1984), inflammation (Wang, 1991b), lymphatic exudation (Tao, 1983) and tetanus (Xiong, 1981). EA stimulation has also been found to produce an analgesic effect, generally called electro-acupuncture analgesia (EAA), in horses (Bossut et al., 1983a and 1984; Sun, 1986; Zhang et al., 1981), cattle (EAA Research Group, 1984; and White et al., 1985), sheep (Bossut et al., 1986; Qin and Yang, 1988), goats (Wang and Tevik, 1990), pigs (Zhu, 1992), dog (Pu et al., 1984; and Janssens and others, 1988), cats (Xie and Wang, 1992), and mice (Cheng and Pomeranz, 1979). However, few controlled studies have been conducted on how acupuncture relieves pain in the horse.

The objective of this study was to determine the effectiveness of acupuncture treatment in relieving pain in the horse, and to determine how acupuncture relieves pain. Supporting results could provide scientific evidence to guide clinical application and selection of more effective acupuncture techniques including electrical frequencies and acupoints. Information from this

work could help define areas where acupuncture can and can not be used in veterinary medicine, and thus, protect horses from medical abuse. The American Veterinary Medical Association (AVMA Guidelines, 1996) now considers acupuncture as an integral part of veterinary medicine; however, the potential for acupuncture abuse is also of concern (AVMA Policy Statements and Guidelines, 1992). Much of this potential abuse is caused by the lack of scientific knowledge about the proper applications of acupuncture. The only way to control this is through scientific studies to define the areas in which acupuncture is efficacious and beneficial.

CHAPTER 2
LITERATURE REVIEW

This literature review focuses on 4 aspects associated with this study.

Pain Threshold and Acupuncture Analgesia

The pain threshold (PT) responses to electricity, heat stimuli, pinprick and pinch were quantified and used to evaluate the effect of acupuncture on analgesia in numerous studies (Bossut et al., 1984; Wu and Huang, 1988; Zhu, 1988; Yu and Zhong, 1985). An increase in PT was interpreted as induced analgesia. Eleven male and 15 female sheep were subjected to EA treatment in the acupoints Yao-pang and San-yang-luo. EA at each acupoint increased ($P<0.01$) the PT in 6 of 7 body areas (Bossut et al., 1986). EA produced an increase in cutaneous PT in 16 of 17 sheep (Qin and Yang, 1988). EA at He-gu (LI-4) produced a significant 12% increase in PT after 10 minutes of acupuncture, and a 27% increase after 60 minutes (Ernst and Lee, 1987). After the new acupoint Yan-chi was stimulated in large animals using EA with 40 to 80 Hz of frequency, PT increased 1.7 times in horses, 1.4 times in mules and 2.4 times in cattle (Zhang et al., 1981). When EA at the Yan-chi group was used for surgeries in 43 large animals, 14 cases were successfully performed, 25 were performed with some degree of animal restlessness and 4 could not be performed (Zhang et al., 1981).

Pu et al. (1984) used EA at the acupoint Tian-men and Bai-hui as analgesia for surgeries in 13 dogs. The surgeries were very successfully performed in 8 dogs, and somewhat successfully performed in 4 dogs and not completed in 1 dog. Guandong Agriculture College (1973) used EA analgesia for laparotomies in 30 head of cattle. Acupuncture analgesia was sufficient for standing surgeries to be performed on 28 head but not effective on 2. White et al. (1985) used EA in the acupoints Bai-hui, Shen-men and Yao-pang for surgery. The acupuncture analgesia was sufficient for standing laparotomies to be performed on 2 unsedated dairy cows.

EA at the ear acupoint, either Er-jian or Qun-hui, produced sufficient analgesia for surgeries to be performed on horses (Yang et al., 1986). EA at the face acupoints Shun-qi and Kuang-xia-kong produced analgesia in cattle (EAA Research Group, 1984).

Bossut et al. (1984) found acupuncture analgesic efficacy varied between 3 groups of acupoints in horses. This phenomenon was called the specificity of the acupoint, that is, different acupoints affect different conditions or symptoms. For example, the acupoint Jiang-ya is only effective for colic while Da-zhui can be utilized for the treatment of high fever. In another EA analgesia study, analgesia was determined on the basis of abdominal midline incisions, entry into the peritoneal cavity, palpation of viscera, and suturing of the incision. In the St-36 and GB-34 EA stimulation group (n=9), 5 showed no pain, 3 moved slightly but remained manageable, and 1 definitely struggled; while in the St-36 and Sp-

6 EA stimulation group (n=8), 1 showed no pain, 1 moved slightly and 6 struggled definitely (Wright and McGrath, 1981). This result indicates that St-6 and GB-34 are much better EA analgesia acupoints than Sp-6. An acupoint's specificity was also reported in pigs (Zhu, 1992). In another clinical study, EA stimulation in the acupoint An-shen induced a strong cutaneous analgesia in pigs, but no analgesia in cattle and sheep (Zhu, 1992). The result indicated that the acupoint's specificity varied among animal species.

Twenty-four male and 10 female dogs were subjected to either He-Ne laser (10 mW) or CO_2 laser (5 W) radiation in the acupoint Bai-hui. PT was increased ($P<0.01$) after 5- to 15-minute He-Ne laser treatment or 30-minute CO_2 laser treatment (Wu and Huang, 1988). He-Ne laser irradiation (6mA and 20mW) induced a significant increase ($P<0.01$) in PT in goats using the acupoints Bai-hui, Shan-gen and Hou-hai (Yu et al., 1982). Chen et al. (1980a) used CO_2 laser radiation in the 6 acupoints of Jia-ji groups for laparotomies in 20 cattle and 30 goats. The surgeries were successfully performed in 42 animals.

Zhu (1989) compared the analgesia effect among different acupuncture methods in rabbits. Conventional needle in Bai-hui and He-Ne laser irradiation in Hou-san-li both significantly increased($P<0.01$) PT in rabbits within 5, 10 and 15 minutes after treatment. However, control treatment did not change ($P>0.05$) PT within the experiment period. Zhou (1989) also found the combination of conventional needle and laser radiation induced a greater increase in PT

(P<0.05) than either alone. This result indicates that a combination of different acupuncture methods may result in a more effective treatment.

When 20 rabbits were subjected to the microwave acupuncture treatment for 10 minutes at the acupoints Bai-hui and Tian-ping, PT was increased (P<0.01) by 171.7% (Yu et al., 1980). Electric stimulation without needle in the acupoint Bai-hui increased PT (P<0.05) in pigs within 15 minutes following treatment (Zhang et al., 1988c). Zhou (1984) used aquapuncture with promacid (100 to 150 mg) at the acupoint Fu-tu as analgesia for surgery in 14 horses. Acupuncture analgesia was sufficient for 10 abdominal surgeries, but not for 4 limb surgeries.

Zhang (1990) used pneumo-acupuncture to induce analgesia for experimental abdominal surgery in cattle. Analgesia was induced from 2 to 5 minutes after 300 to 400 ml of air was injected into Bai-hui and 200 to 300 ml of air was injected into An-fu and Tian-peng. Acupuncture analgesia lasted 3 to 4 hours. Five abdominal surgeries were successfully performed in 3 cattle using this acupuncture analgesia technique.

Mechanical dolorimeter has also been used to measure PT (Farber et al., 1997). The meter was pressed against a point on the body surface using a constant and homogeneous pressure until pain was referred. After EA stimulation at LI-4, PT increased by 77 % at acupoint LI-5, but only by 9% and 6% at the non-acupoint 15 mm internally and externally lateral to LI-5; PT increased 70% at acupoint LI-11, only 6% and 7% at the non-acupoint lateral to

LI-11; PT increased 81% at the same side of LI-20; PT increased 108% at the other side of LI-20 (Farber et al., 1997). This study confirms the importance of the meridians, pathways where chi (energy) flows through the body.

Clinical Research on Relief of Pain by Acupuncture

Various types of acupuncture techniques have been reported to treat clinical conditions in horses. Tables 2-1 to 2-7 lists the clinical results of acupuncture for treatment of back pain, joint contusion, muscular atrophy, rheumatic pain, tendinitis, laminitis and colic in horses.

Mechanism of Acupuncture on Pain Relief

Traditional Acupuncture Model for Pain

Based on the observation that stimulation of specific areas on the body surface could relieve pain and internal discomfort, the ancient Chinese people called these specific spots "Shu-Xu", or acupoints. Then they discovered that there was a close relationship between some acupoints. From point to point, they organized them into a line and pathway, which is called "Jing-mai". Jing-mai means channel or meridian. There are 14 meridians in the body. A classical acupuncture point is located on a meridian.

According to traditional Chinese medical philosophy, each major channel is associated with a specific internal viscera and also reflects the physiological and pathological conditions of that organ. When an organ is affected with pathological factors, some acupoints on the related meridian may become

Table 2-1 Acupuncture for treatment of back pain in horses

Type of Acupuncture	Clinical results (# of case)			Reference
	Improved	failure	Total cases	
Conventional needling	13	2	15	Martin & Klide, 1991
Aquapuncture using vitamin B1 etc.	139	16	155	Martin & Klide, 1987a
	49	1	50*	Lu, 1983
	50	0	50*	Tang, 1983
	7	1	8	Gansu Institute, 1976a
PneumoA **	11	1	12	Gansu Institute, 1976b
EA ***	14	0	14	Pei, 1981
Fire-needling	49	2	51*	Zhao, 1982
Vinegar-Liquor hot Moxibustion	45	0	45*	Wang, 1992
	5	0	5*	Fu, 1992
Laser	11	4	15	Martin & Klide,1987b
Total number of cases	393 (94%)	27 (6%)	420	

*: Mixed animals;

** PneumoA: pneumo-acupuncture;

*** EA:electro-acupuncture

Table 2-2 Acupuncture for treatment of paralysis and paresis in horses

Location of paralysis	Type of acupuncture	Clinical results (# of case)			Reference
		Improved	Failure	Total	
Radial nerve	EA+AquA *	4	1	5	Wang & Hu, 1983
	AquA	5	0	5	Wang, 1991a
Suprascapular	PneumoA	16	1	17	Liang, 1980
Hindlimb	EA	1	0	1	Dan et al., 1995
Glossopha-ryngeal	EA	2	0	2	Shi, 1984
Bladder	EA	5	3	8	Sichuan-shehong1976
Facial nerve	EA	9	0	9	Guo, 1984
	EA + AquA	4	1	5	Wang&Hu, 1983
	AquA	3	0	3	Bai, 1987
	A-shi + Herb	9	0	9	Wang,1991a
	Embedding	1	0	1	Ma, 1981
Total numbers of cases		59 (91%)	6 (9%)	65	

*AquA: aquapuncture

Table 2-3 Acupuncture for treatment of lameness due to rheumatic pain

Type of Rheumatism	Type of acupuncture	Clinical results (# of case)				Reference
		Sound	Improved	Failure	Total	
Four limbs †	EA + CA*	13	11	1	25	Liang, 1982
	EA	174	21	3	198	Li, 1993
Hindlimb †	VLHM** +AP***	0	122	0	122	Meng et al., 1993
Hindlimb in horses	Warm-needling	0	8	0	8	Tao & Wang, 1984
	AquA	0	6	0	6	He et al., 1982
General rheumatism in horses	CA + SS****	9	3	2	14	Liang, 1984
	CMM*****	0	19	3	22	Ji & Du, 1990
In cattle	AquA	0	20	1	21	Tan, 1984
Limb&neck	VLHM +CA	0	19	2	21	Zhu, 1987
Cervical	EA	23	0	4	27	Zhang, 1980
Rheumatism †	EA	143	0	10	153	Sichuan-shehong,1976
Total numbers of cases		362 (59%)	229 (37%)	26 (4%)	617	

†: Mixed animals

* CA: Conventional acupuncture

** VLHM: vinegar-liquor hot moxibustion

*** AP: acupressure

****SS: sodium salicylate

*****CMM: Chinese medicine moxibustion

Table 2-4 Acupuncture for treatment of lameness due to joint contusion

Joint contusion	Type of acupuncture	Clinical results (# of case)				Reference
		Sound	Improved	Failure	Total	
Shoulder or hip/stifle	He-Ne laser	0	36	2	38	Yang, 1984
	HA*+Herbs	34	0	1	35	Guo & Li, 1983
	EA	6	0	0	6	Jiangxi-xingzi,1976
Shoulder	CA+massage	1	0	0	1	Wang, 1986
Hip+stifle	HA		7	0	7	Jing et al.,1988
Carpus	Laser+AquA	1	0	0	1	Xie & Wang, 1991
stifle + metacarpus	Laser	4	0	0	4	Zhao et al., 1983
Fetlock	HA	0	106	0	106	Li, 1986
	CA+Herbs	6	0	0	6	Liu, 1991
	HA	0	63	0	63	Zhang, 1988b
Forelimb	AquA+HA	83	2	0	85	Sun & Gao, 1989
	AquA	0	61	1	62	Chen, 1989
Forelimb or hindlimb	AquA+Herb	22	0	1	23	Ming & Gao, 1989
	AquaA	37	0	10	47	Bai et al., 1989
	AquA+Herbs	136	15	10	161	Liu et al., 1990
	EA	2	0	1	3	Zhang, 1980
Total numbers of cases		332 (51%)	290 (45%)	26 (4%)	648	

* HA: hemo-acupuncture

**CM: conventional medicine .5%prednisolone and 2% procaine

Table 2-5 Acupuncture for treatment of lameness due to muscular atrophy

Location of muscular atrophy	Type of acupuncture	Clinical results (# of case)				Reference
		Sound	Improved	Failure	Total	
Shoulder in horse	PneumoA	46	0	4	50	Su, 1982
	EA+PneumoA	1	0	0	1	Xie & Wang, 1991
	Cupping	0	1	0	1	Zhang, 1988a
Shoulder in cattle	PneumoA	7	0	1	8	Zhang, 1981
	PneumoA	17	0	5	22	Chen, 1984
Hip in horse	EA+PneumoA	1	0	0	1	Zhang, 1986b
Total numbers of cases		72 (87%)	1 (1%)	10 (12%)	83	

Table 2-6 Acupuncture for treatment of tendinitis and laminitis in horses

Type of lameness	Type of acupuncture	Clinical results (# of case)				Reference
		Sound	Improved	Failure	Total	
Tendinitis	Soft-burning	1	0	0	1	Xie & Wang,1991
	Magnetic	2	0	0	2	Bai et al., 1982
Laminitis	HA	39	0	6	45	Wang, 1990
	EA,HA+AquA	0	0	3	3	Xie (unpublished)
Total numbers of cases		42 (82%)	0	9 (8%)	51	

Table 2-7 Acupuncture for treatment of colic in horses

Type of acupuncture	Clinical results (# of case)			Reference
	Clinical relief	Failure	Total	
EA	89	11	100	Zhu, 1979
HA+/orJiang-ya	284	22	306	China Agriculture Academy Vet Inst, 1960
HA +/or Jiang-ya	878	65	943	Lu, 1973
EA	16	5	21	Gao, 1987
Fire-needling	27	8	35	Zhang, 1989
EA	8	0	8	Zhou, 1992
HA + EA	285	0	285	Sun, 1986
HA +/or Jiang-ya	51	6	57	Yang, 1983
Total numbers of cases	1638 (93%)	117 (7%)	1755	

tender or show other signs of abnormality. When the acupoints are stimulated by acupuncture, the affected organ could recover. This traditional philosophy was similar to the neurophysiologic concept of viscero-somatic or somato-visceral reflexes (Hwang and Egerbacher, 1994). In one study, acupuncture stimulation at St-36 induced a decrease in sympathetic renal nerve activity (RNA) and mean arterial blood pressure (MAP) in rats, while acupuncture stimulation only at the skin of the acupoint alone did not induce any change of MAP and RNA (Ohsawa et al., 1995). This result indicates that anatomic structure of acupoint consists of the deeper tissues rather than only skin. However, precise anatomic structure of an acupoint needs to be carefully studied.

Numerous studies show the anatomic and physiological differences between acupoint and non-acupoint sites. Acupoints are located in the areas with a higher concentration of mast cell and free nerve endings (Hwang and Egerbacher, 1994). Acupoints and meridians were found with characteristics of lower skin electric resistance and higher percussion sound (Yu et al., 1994). Analgesia induced by acupoint stimulation is naloxone reversible, while analgesia caused by stimulation of non-acupoints (abdominal muscles) after lesion of analgesia inhibitory system is dexamethasone reversible (Takeshige, 1985). Zhou and Xi (1986) compared the transcutaneous nerve stimulation analgesia (TNSA) with EA analgesia (EAA) at the acupoints He-gu and Wai-guan, and found that naloxone partly reversed EAA but not TNSA. TNSA was blocked partly by either deep or intradermal injection of procaine, while EAA was

almost completely blocked by deep injection but not by intradermal injection of procaine.

An additional traditional Chinese medical philosophy is that health of the body depends on Chi (qi, vital energy, or life energy force). Physiologically, Chi flows all over the body through the meridian pathway all the time. When the flow of Chi is interrupted by the pathological factors, an imbalance or blockage of the body's Chi may occur. In Traditional Chinese Medicine"s (TCM) perspective, pain is interpreted as the blockage of Chi or no free flow of Chi. "Where there is no free flow of Chi, there must be pain" (Yu, 1984) (figure 1-1). Acupuncture stimulation is thought to reduce blockages along the meridians, free the flow of Chi, enable the body to heal itself and return to a state of homeostasis. "Where there is free flow of Chi, there is no pain" (Yu, 1984) (figure 1-2). The traditional Chinese medical pain model was illustrated in figure 1-1 and acupuncture pain relief was illustrated in figure 1-2.

Inhibition or Reduction of Transduction and Transmission

The perception of pain involves 4 phases: transduction, transmission, modulation and perception. Acupuncture analgesic effect may be related to one or more of these 4 pain processing phases.

One hypothesis proposed that stimulation of specific acupoints can block pain sensations before they reach the central nervous system (Snader, 1993). Numerous studies have shown that the lightly myelinated Aδ fiber is consistently considered the most dominant in mediating acupuncture, followed by

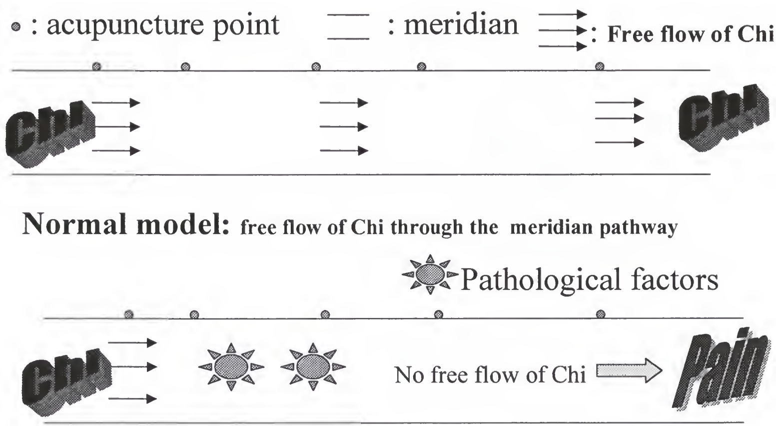


Figure 1-1 Traditional Chinese medical pain model

"Where there is no free flow of Chi, there must be pain"

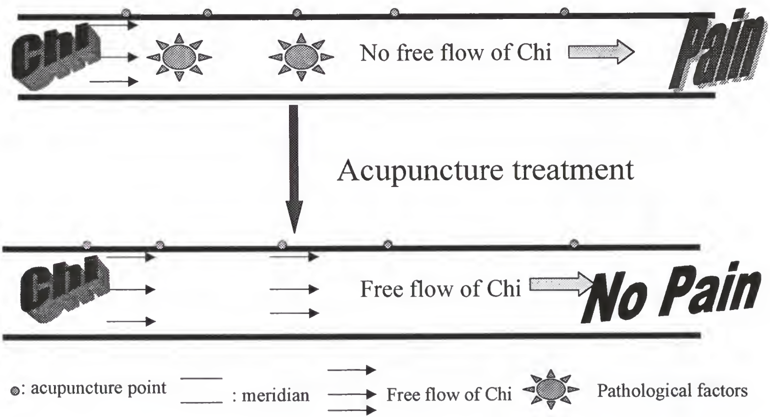


Figure 1-2 Traditional acupuncture pain relief model

"Where there is free flow of Chi, there is no pain"

unmyelinated cutaneous C fibers (Kendall, 1989). Aδ and C fibers are also considered nociceptors (nociceptive afferents), or noxious receptors. The repetitive stimulating pain depends on Aδ fibers, which can be blocked by pressure. The intense prolonged pain depends on C fibers, which can be blocked by local anesthetic. The afferent nociceptive Aδ and C fibers project to lamina I of the spinal cord. The acupuncture signal, on the other hand, is transmitted to lamina I of spinal cord by Aδ and C fibers at the same time when acupuncture is used for a pain-producing disease. A segmental spinal inhibition of nociceptive inputs based on the gate control theory results in an immediate, short-lasting, segmental and non-opioid analgesia (Ernst and Lee, 1987).

Yonehara et al. (1992) found that the tooth pulp stimulation (TPS) evoked an increase in release of immunoreactive substance P (iSP) in trigeminal nucleus caudalis (Vc-I,II) in rabbits. This increase was inhibited by EA at ST-36 in 9 out of 13 rabbits. The potentials evoked by TPS consisted of fast and slow components. The slow potential, reflecting the excitation of Aδ fibers, was significantly inhibited by CP-96,345, an SP antagonist. The slow potential evoked by TPS was also inhibited by EA stimulation in 8 out of 11 animals. The trigeminal nucleus is considered to be a relay for impulse transmission from the tooth pulp afferents, including Aδ and C fibers, to the tract cells of the ascending pathways to higher levels of the brain. SP might be involved as a primary afferent transmitter in the transmission of dental pain messages (Yonehara et al., 1992). Thus, Acupuncture may inhibit SP release from the primary sensory

afferent fibers and relay neurons and consequently block onward transmission of the pain impulse to the high levels of the central nerve system.

Acupuncture stimulation at ST-36 induced a decrease in sympathetic renal nerve activity (RNA) and mean arterial blood pressure (MAP) in rats under the deep anesthetic condition (Ohsawa et al., 1995). But, acupuncture stimulation at the skin of acupoint alone did not induce any change of MAP and RNA. This suggests that anatomic structure of the acupoint consists of the deeper tissues rather than skin.

Neuroendocrine Response–Modulating Pain

The word opium is derived from the Greek word meaning "juice" perhaps because the first use of the poppy plant was its juice. Opium is the dried powdered mixture of 20 alkaloids which is obtained from the unripe seed capsules of the opium poppy plant (Ferrante, 1993) . The term opiate refers to any agent which is derived from opium such as an alkaloid. The major phenanthrene alkaloids derived from opium include morphine, codeine and thebaine. The term opioid refers to all endogenous and exogenous (including natural or synthetic) substances that possess morphine-like properties (Ferrante, 1993). Morphine is the prototypic opioid. The endogenous opioids work as one part of an endogenous analgesic system that modulates nociception (Katz and Ferrante, 1993). They are derived from 3 precursor molecule sources: proenkephalin A, pro-opiomelanocortin and prodynorphin (proenkephalin B). Enkephalins including met- and leu-enkephalin are derived from proenkephalin

A and found in high density in areas of the central nervous system: the periaqueductal gray(PAG), the rostroventral medulla, and laminae I, II, V, and X. Dynorphins including dynorphin and α-neoendorphin are derived from prodynorphin or proenkephalin B. Even though their anatomic location is similar to enkephalins, they are not potent analgesics (Ferrante, 1993). Numerous studies indicate enkephalins get involved in acupuncture analgesia (Zhu, 1988, Zhou, 1982 and Han, 1997). D-phenylalanine (DPA) is known as an enkephalinase. DPA administration increased ($P<0.01$) acupuncture analgesic effect by 35% (Kitade et al., 1990) and prolonged the acupuncture analgesia over 2 hours (Kitade et al., 1988).

β-endorphin is a 31 amino acid carboxyterminal and the most potent of the endogenous opioids. It is derived from a series of proteolytic cleavages of pro-opiomelanocortin. β-endorphin was originally isolated from the pituitary gland and has been demonstrated to be released into the peripheral circulation after stress or acupuncture (Guillemin et al., 1977 and Nappi et al., 1982). The high concentrations of β-endorphin are found in the hypothalamus, PAG, locus coeruleus and preoptic area (Ferrante, 1993 and Hong et al., 1977).

Opioids interact with several types of receptors in the brain and other tissues. Most endogenous opioids bind to μ-receptors, or morphine-preferring receptors. The prototypic endogenous ligand is β-endorphin while the prototypic exogenous ligand is morphine. μ-receptors are found specifically in areas of the brain that are intimately involved with opioid-induced analgesia: PAG, the

nucleus raphe magnus, and the medial thalamus (Quirion et al., 1983). κ- and δ-receptors are localized to the spinal cord. Thus, activation of μ-receptors is largely responsible for supraspinal analgesia (Ferrante, 1993).

Endogenous morphine-like substances. The endogenous opioid peptides were discovered by Hughes in 1975 (Hughes, 1975). Since the acupuncture effect can be reversed by naloxone, a specific opiate antagonist (Mayer et al., 1977), the endorphin system has been generally considered a possible pathway for acupuncture analgesia. Cepeda and Carr (1993) reviewed the neuroendocrine response to pain. ACTH and β-endorphin are found to be derived from the same precursor molecule, pro-opiomelanocortin (POMC). POMC undergoes a series of ordered proteolytic cleavages and modifications in the corticotrophin of the anterior pituitary to yield ACTH and β-lipotropin (β-LPH). β-LPH, in turn, gives rise to daughter molecules, including β-endorphin (Cepeda and Carr, 1993). It appears that at least 3 separate endogenous opioid neuronal systems are present in the brain: an enkephalin family with components similar to those found in the adrenal; a β-endorphin family, and a dynorphin family (Watson et al., 1982). β-endorphin might produce its analgesic effects by suppressing substance P (SP) release in the spinal cord. Methionine enkephalin blocks release of SP, which may be related to what closes the gate in the spinal cord pain transmission system (Lumb and Jones, 1984).

Acupuncture was found to induce an increase in endogenous morphine-like substances (EMLS) in the cerebrospinal fluid (CSF) drained from the

cerebroventricle in patients suffering from brain tumors (Zhang et al., 1980), and in serum EMLS in patients with soft tissue pain, acute appendicitis and peri-arthritis (Xi et al., 1983). In a study using the lip twitch, a form of acupuncture known as acupressure, β-endorphin release was investigated in the horses. The use of lip twitch resulted in a doubling of plasma β-endorphin levels in as little as 5 minutes after application of the twitch (McCarthy et al., 1993). There was a linear correlation between increases in β-endorphin and the body's ability to tolerate pain (Hamra et al., 1993). EA stimulation induced a large amount of β-endorphin released into the peripheral blood (Malizia et al., 1979). Similar to micro-iontophoretic opiates, morphine and etorphine, EA produced a strong inhibition on the spontaneous activity of the periaqueductal gray (PAG) neurones, and this inhibition could be reversed by iontophoretic naloxone (He and Dong, 1983). PT was increased significantly in rabbits after 10-minute acupuncture stimulation at LI-4 and TB-5. At the same time, acupuncture produced a significant increase in the release of leucine-enkephalin and β-endorphin in the preoptic area (Wu et al., 1995).

Injection of protein-A purified antibodies against Met-enkephalin or β-endorphin into the periaqueductal gray (PAG) was shown to decrease the analgesic effect of EA in rabbits (Han et al., 1984). Antibodies of Met-enkephalin were found to be active at the spinal level whereas antibodies against β-endorphin were without effect, which is in agreement with a rich enkephalinergic

innervation and absence of β-endorphin-containing fibers in the spinal cord (Han et al., 1984).

Both acupuncture and morphine analgesic effects increased progressively during ontogenetic development in rabbits (Zhou et al., 1982). Acupuncture analgesia effect was not found in 2-, 7- and 14-day-old rabbits. However, significant acupuncture analgesia was found in 21-day-old rabbits and acupuncture analgesia in 28-day-old rabbits was almost the same as that in the adults. Morphine analgesia was not found in 2- and 7-day-old rabbits, but was found in 14- and 28-day-old rabbits. Both acupuncture analgesia and morphine analgesia were reversed by administration of naloxone.

Plasma levels of β-endorphin, β-lipotropin and ACTH increased significantly in the volunteers after EA stimulation at the acupoints St-36, LI-4 and P-7, while conventional needles at the same acupoints without electric stimulation did not change these parameters (Nappi et al., 1982). This result coincided with the clinical findings in that EA was more effective than conventional acupuncture (Liang, 1982).

D-phenylalanine (DPA), an enkephalinase inhibitor, is known to block the activity of carboxypeptidase, an enzyme which degrades enkephalins (Kitade et al., 1990). DPA was found to potentiate the pain relief induced by acupuncture in animals and humans and produce naloxone-reversible analgesia in mice (Ehrenpreis, 1985). Kitade et al.(1988) found DPA a potentiated acupuncture-induced increase in PT in healthy volunteers. DPA increased acupuncture

analgesia results by 26% in 30 patients with chronic low back pain and by 35% in 18 patients with tooth extraction (Kitade et al., 1990). A pressor blood pressure response was elicited by strong electric shock stimulation at the front paw in rabbits. EA inhibited the pressor response and this inhibition was readily blocked by naloxone (Wang et al., 1994).

However, Szcudlik and Lypka (1983) found that EA resulted in a significant decrease in plasma beta-endorphin-like immunoreactivity in the healthy volunteers, and plasma ACTH levels did not change after acupuncture. They suggested that the reaction of the beta-endorphin system to acupuncture stimulation in humans did not involve pituitary hypersecretion and the increase of beta-endorphin binding to the tissue receptor sites seems to be responsible for the peripheral β-endorphin decrease after acupuncture. Human clinical studies also showed that acupuncture analgesia might not be mediated by the endorphin system (Kenyon et al., 1983). After acupuncture treatment, total pain relief was observed in 8 out of 30 patients with pain, varying levels of pain relief in 20 patients and no pain relief in 2 patients. Immediately after injection of 1 ml (400 micrograms) of naloxone, or 1 ml of 0.9% sodium chloride, no change in pain relief was recorded in patients. After 30 minutes following injection, the degree of pain relief was unchanged in 27 patients, worse in 2 patients (both had saline injections) and improved in 1 patient who had naloxone injection. These results indicated that endorphin may not be the only factor in acupuncture analgesia, or that naloxone does not block all opiate receptors.

Another study showed that intravenous injection of naloxone did not affect the decrease in sympathetic renal nerve activity (RNA) and mean arterial blood pressure (MAP) induced by acupuncture in rats under the deep anesthetic condition (Ohsawa et al., 1995). This indicated that the acupuncture effect is a reflex response under deep anesthetic condition and endogenous opioids may not be involved.

<u>ACTH and cortisol</u>. Stimulation of acupoints triggers the pituitary gland to release adrenocorticotropic hormone (ACTH), which stimulates the adrenal glands to release cortisol into the blood stream. Serum cortisol is a natural steroid anti-inflammatory agent which acts to reduce inflammation and pain in affected areas (Smith, 1992). EA stimulation induced large amounts of ACTH to be released into the peripheral blood (Malizia et al., 1979).

EA produced a 33% increase in PT in rats, and after dexamethasone injection, the acupuncture-induced increase in PT dropped by 11% (Liu et al., 1988). Thus, dexamethasone administration reduced acupuncture analgesic effect. Dexamethasone could block acupuncture analgesia by inhibiting release of β-endorphin from the pituitary. Some evidences demonstrated that dexamethasone inhibits secretion of both β-endorphin and ACTH from the pituitary. Takeshige (1985) found out that acupuncture analgesia was naloxone reversible, non-acupoint analgesia was dexamethasone reversible, and stress-induced analgesia by low frequency electrical shock was both naloxone and dexamethasone reversible. All 3 types of analgesia were related to the

descending pain inhibitory system, a common pathway for analgesia. This pathway was found in the arcuate nucleus (dopaminergic), ventromedian nucleus of the hypothalamus, raphe nucleus (serotonergic), reticular gigantocellular nucleus (noradrenergic) and reticular paragigantocellular nucleus.

Substance P (SP). SP seems to be important in mediating effects of EA. Injection of antibodies against SP into the PAG caused decrease of the acupuncture analgesia whereas intrathecal administration of Fab-fragment SP antibodies caused a marked potentiation (Han et al., 1984). However, repeated EA at UB-11 and UB-54 significantly increased SP, neuropeptide Y and neurokinin A in the hippocampus and neuropeptide Y in the occipital cortex in rats (Bucinskaite et al., 1994).

Other endocrine responses. PT was increased significantly in rabbits after 10-minute acupuncture stimulation at LI-4 and TB-5. At the same time, acupuncture produced a significant decrease in release of noradrenaline in preoptic area (Wu et al., 1995).

Acupuncture stimulation was found to increase the blood concentration of free tryptophan, the serotonin precursor, in healthy volunteers subjected to ketamine (Costa et al., 1982). Serotonergic neurons could be involved in analgesia by acupuncture. These serotonergic neurons consists of the so-called "negative feedback-loop system" , which could activate a descending system

with inhibitory effects on spinal cord pain-transmitting neurons, resulting in analgesia (Scherder and Bouma, 1993).

Animal experiments revealed that the activity of the cholinergic system in certain brain areas enhanced acupuncture analgesia while that of the dopaminergic system attenuated acupuncture analgesia (He and Xu, 1981). The acetylcholine (Ach) contents in the ventricular perfuse was significantly increased with the elevation of PT after acupuncture stimulation (He and Xu, 1981). The main source of Ach in the cerebrospinal fluid of the lateral ventricle is from caudate nucleus (CN) and the septal area (SA). Scopolamine, a cholinergic blocker, was found to readily block acupuncture analgesia (He and Xu, 1981).

Metoclopramide, having antidopamine and anticholinesterase actions, was found to be synergic with acupuncture analgesia both in the laboratory and clinical studies (Xu et al., 1983). This result indicates that metoclopramide can be used as an adjuvant to improve acupuncture analgesia.

Auriculo-acupuncture electro-stimulation with frequencies of 15 and 100 Hz induced an analgesic effect in rabbits that expressed a decrease in the amplitude of a cortical somatosensory evoked potential in response to tooth pulp electro-stimulation (Fedoseeva et al., 1990). They also found intracerebroventricular saralasin (angiotensin II antagonist) injection abolished or blocked the acupuncture analgesia at 100 Hz, but not at 15 Hz frequency. Intravenous injection of naloxone abolished the effect of acupuncture analgesia at 15 Hz, but not at 100 Hz. This result indicated the neuropeptide angiotensin II

was an antinociceptive factor in dental peptide analgesic mechanism induced by auriculo-acupuncture stimulation at 100 Hz frequency.

Takeshige and Sato (1996) used the reduction of twitch height of tetanized gastrocnemius in guinea pigs as a model of pain. The recovery of twitch height was considered as pain relief. Muscle pain relief might be induced by recovery of circulation due to the enhanced release of acetylcholine as a result of activation of the cholinergic vasodilator nerve endings innervated to the muscle artery. Needling at the tetanized gastrocnemius muscle facilitated recovery from the reduced twitch height due to tetanic stimulation. This needling recovery effect was abolished by intravenous injection of atropine. Although a simple cut of the sciatic nerve innervated to the gastrocnemius muscle did not influence the needling effect, denervation 2 weeks after the nerve cut, abolished the needling effect. The needling effect was also abolished by capsaicin, which depletes SP and the calcitonin-gene related peptide (CGRP). Intraarterila injection of either SP or CGRP recovered the reduced twitch height. These results indicate that needling to the muscle most likely stimulates many kinds of the sensory nerve endings such as CGRP containing nerve endings. These CGRP containing nerve endings might innervate and stimulate the cholinergic nerve endings to accelerate the release of acetylcholine and dilate the artery in the muscle by axon reflex, since denervation and atropine abolished this needling recovery effect (Takeshige and Sato, 1996).

A main pathway of acupuncture analgesia (AA) is via the release of endogenous opioid peptides. The exogenous opiate has a potentiating effect on AA, while some peptides, including cholecystokinin (CCK), angiotensin II and orphanin FQ (OFQ) have anti-AA effect. OFQ is a newly discovered 17-amino-acid peptide, which is sometime called "nociceptin" (Zhu et al., 1996). Intracerebroventricular or intrathecal administration of OFQ induced hyperalgesia in rat tail-flick model and showed a dose-dependent antagonizing effect against AA. OFQ anti-AA effect was abolished by intracerebroventricular administration of antisense oligonucleotide (ASO), which could block the synthesis of OFQ receptor. ASO was also found to enhance AA (Zhu et al., 1996).

Neuro-Structure for Acupuncture Analgesia

Electrical stimulation of the nucleus reticularis paragigantocellularis lateralis (RPGL) increased the PT and enhanced the AA in rats (Li et al., 1995). Lesion of the RPGL reduced the acupuncture analgesic effect. Microinjection of naloxone into the RPGL partially reversed the AA effect and the naloxone reversal was dose-dependent. EA also produced a significant increase in the release of leu-enkephalin and β-endorphin from the RPGL (Li et al., 1995).

The immediate early gene, c-fos encodes a nuclear phosphoprotein, Fos which has been proposed to be a third messenger to regulate the expression specific target genes (Zhang et al., 1996). Wang et al. (1995) used an immunohistochemical technique to detect the expression of c-fos like protein in

rats. They found that in noxious stimulation and acupuncture + noxious stimulation groups an increased amount of Fos-like immunoreactive (FLI) cell nuclei appeared in the dorsal horn of lumbar and sacral spinal cord (Laminae I, II, V and X), nucleus reticularis lateralis, nucleus reticularis paragigantocellularis lateralis, area A1, nucleus tractus solitarii, nucleus raphe magnus, locus coeruleus, nucleus raphe dorsalis and periaqueductal grey (PAG). In the control group, no obvious c-fos expression was shown in the structures mentioned above. It was shown however that, in the lumbar and sacral spinal cord, the number of FLI nuclei in the acupuncture + noxious stimulation group was more than that in the noxious stimulation group. The number of FLI nuclei in acupuncture+noxious stimulation group was less than noxious group at the locus coeruleus and the ventral part of PAG. These results imply that acupuncture and noxious stimulation may activate the intrinsic pain modulating system through the spinal dorsal horn, then suppress the c-fos expression induced by noxious stimulation in the locus coeruleus and ventral part of the PAG. Using techniques of in situ hybridization and immunocytochemistry, Zhang (et al., 1996) demonstrated that EA stimulation at ST-36 activated the coexistence of Fos protein and proopiomelanocortin (POMC) mRNA in the hypothalamic arcuate nucleus (Arc) cells in rats. The coexistence of Fos protein and POMC mRNA poses the possible relationship of Fos regulating the POMC expression.

The local cerebral metabolic rate of glucose (LCMRG) by Sokoloff's 2-deoxyglucose auto-radiographic quantitative analysis was used to determine the

neurological structure of AA (Jia et al., 1994). The LCMRG at the spinal thoracic and lumbar dorsal horns L1-L3, nucleus raphe magnus (NRM), nucleus reticular gigantocellularis (RGI), and periaqueductal gray (PAG) was considerably increased while at the T7-T9, locus coeruleus (LC), and habenulae lateralis (HL) of thalanum significantly decreased in EA group. In the processes of AA, the excitation of NRM, RGI and PAG could weaken the action of LC and HL. The input of acupuncture and/or pain itself via the ventrolateral funiculi (VLF) activated the descending pathways through the dorsolateral funiculi (DLF) from supraspinal structures of endogenous pain modulatory system which inhibited the pain input. All these nuclei could potentially be the key in AA.

Relationship Between Body Surface and Internal Organ

Low skin impedance. Low skin impedance was found on the specific acupoints on Guan-yuan-shu, Qian-shu, Liu-mai and Hou-hai in 3 cattle with gastrointestinitis (Feng, 1990). Low impedance on the acupoints Guan-yuan-shu, Wei-shu, Pi-shu and Da-chang-shu was found in a mule with indigestion. Following stimulation of these highly-sensitive acupoints by EA daily for 3 days, the mule improved after the second treatment and was clinically recovered after the third treatment (Feng, 1990).

High skin temperature. Feng (1990) found the skin temperature of specific acupoints increased when animals suffered from an internal problem. An increase in skin temperature current was found on the acupoints Guan-yuan-shu, Qian-shu, Liu-mai and Hou-hai of 3 cows with gastrointestinitis. The skin

temperature on these acupoints was 0.05 to 0.2 ^{0}C higher than on other acupoints in these cows. The skin temperature was 0.3 to 0.5 ^{0}C higher on the acupoints San-jiang, Jiang-ya and Er-jian than other acupoints in 4 horses with colic. Colic was resolved within 15 to 60 minutes after these highly-sensitive acupoints were stimulated with acupuncture.

Other Physiological Responses to Acupuncture

When EA increased cutaneous PT (analgesia) in sheep, it did not result in any statistically significant ($P>0.05$) change of blood measurements which included RBC, WBC, Hb, PCV, pH, PCO_2, PO_2, HCO_3^-, Total CO_2 and plasma K^+ (Wang and Tevik, 1990). These results suggest that EA analgesia induces no physiologically detectable side-effects and could be used for surgery in aged and weak animals.

Although EA at Qiang-feng and Bai-hui did not result in significant changes ($P>0.05$) in respiratory rate, pulse rate, Hb and RBC, it induced a significant increase ($P<0.05$) in WBC and neutrophil counts (Li et al., 1985). EA at GV-16 and GV-8 remarkably reduced the cerebral infarction volume in rats subjected to transient focal cerebral ischemia induced by 2 hours of middle cerebral artery occlusion. EA also decreased the elevated extracellular aspartate level induced by ischemia and enhanced the elevation of taurine. EA did not change extracellular concentration of glutamate (Zhao and Cheng, 1997).

EA stimulation at St-36 in combination with Sp-6 or GB-34 induced a general tendency for heart rate and respiratory rate to decrease in dogs during the 40 minutes of observation, as compared with that of dogs receiving no acupuncture treatment which had slight increases in heart rate and respiratory rate. However, the differences were not statistically significant (Wright and McGrath, 1981). Even though there were statistically significant decreases in systolic blood pressure during electro-stimulation of nonacupuncture points alone and of St-36 alone, the magnitude of these decreases was small, ranging from 3.75 to 4 mm Hg (Wright and McGrath, 1981).

Evaluation of Pain and Pain Model in Horses

The ultimate aim of equine medicine is to relieve animal pain and suffering. Equine practitioners may accomplish this aim without recognizing pain, however, it is not possible for them to assess the effectiveness of treatments without defining the expression of pain. Thus, pain models have been developed for the evaluation of pain and analgesic effect in horses. Many pain models have been reported to assess the effectiveness of analgesics and sedatives in the horses (Matthews, 1992; Pippi and Lumb, 1979; Kamerling et al., 1984; and Kamerling et al., 1985). The pain models used in horses can be divided into invasive and noninvasive. The invasive models require surgery, implantation of a device, or permanent alteration of the horses before experimental study. They include dental dolorimetry (Brunson et al., 1987), inflatable rubber balloons (Lowe, 1992), implanted heating element (Pippi and Lumb, 1979) and tendon

injury (Chambers et al., 1993) models. In the dental model, a monopolar electrode was surgically implanted into the tooth pulp of the upper canine tooth. A single stimulus of 2-ms duration, repeated at equal to or greater than 20-s intervals, was used to elicit a head lift response. The lowest current level that produced 3 positive head lift responses was considered as the tooth pulp pain thresholds (TPPT) of the horse (Brunson et al., 1987). This technique stimulates the tooth pulp nerve, which consists of A-δ and C-fibers, thus, a pain-only afferent is involved. The analgesic effectiveness of xylazine and xylazine-opioid combinations has been evaluated using this model (Matthews, 1992). In the balloon model, a rubber balloon was passed into the cecum through a surgically implanted cannula. When the balloon was distended, stretching the cecal wall until the pressure reached approximately 30 mmHG, the horse began to show typical signs of colic. Responses such as anorexia, restlessness, looking at its flank, pawing, stretching the body, crouching, stretching the neck and curling the upper lip, vocalizing, lying down, getting up again, and lying down and rolling, were scored by the observer (Lowe, 1992). The principal disadvantage of this model was that the scoring method was subjective (Kamerling et al., 1989). Regular inflation and deflation or filling the balloon with fluid instead of air appeared to produce more consistent results (Matthews, 1992). This model has been used to test the colic-attenuating effects of xylazine, fentanyl, meperidine, oxymorphone, pentazocine, flunixin, dipyrone and detomidine (Kalpravidh et al., 1984 and Matthews, 1992). In the heating element

model, a direct current of 12.5 A at 12 V was passed from a power supply to a heating device surgically implanted on the midlateral surface of the radius (Pippi and Lumb, 1979). Response time was measured using an accelerometer attached to a recorder for accurate measurement of time to limb motion. This model was used for evaluation of the moderation of deep pain by xylazine, fentanyl, meperidine, methadone, oxymorphone and pentazocine (Matthews, 1992). In the tendon injury model, collagenase was injected into the superficial digital flexor tendon of the left foreleg on four occasions at weekly intervals. After the final injection of collagenase, the tendon damage was assessed ultrasonically, the circumference of the legs was measured and the degree of lameness at the trot was scored on a visual analogue scale (Chambers et al., 1993). This model has been used to evaluate the analgesic effects of detomidine in thoroughbred horses (Chambers et al., 1993). Although all the models mentioned above have been used to evaluate the effectiveness of analgesics and sedatives in horses, few reports on physiological changes induced by these pain models alone have been found.

Noninvasive pain models do not require surgery or permanent alteration of the horses, thus, they are more commonly used. They include the cutaneous stimulus (Bossut et al., 1983a), the light beam (Hamra et al., 1993; Goetz and Comstock, 1986) , modified balloon (Schatzmann et al., 1992) and reversible lameness (Merkens and Schamhardt, 1984) models. In the cutaneous model, pinprick and pinch were used as noxious stimuli on the skin to elicit a withdrawal

response. This is the simplest model to test superficial pain in horses. It has been used to evaluate the acupuncture analgesic effect in horses (Bossut et al., 1984) and sheep (Bossut et al., 1986). Even though this model is simple, repeated stimuli can produce a conditioned response due to anticipation (Matthews, 1992). In the light beam model, focused radiant light is used as a superficial pain stimulus and is directed onto the pastern (Harkins et al., 1996) or wither (Kamerling et al., 1985) to elicit the classical withdrawal reflex. When the horses withdraw their hoof or have a skin-withdrawal reaction on the back, they are considered to have signaled their perception of pain after a pain stimulus is applied. These methods have been used to evaluate the effectiveness of flunixin, carprofen, procaine, cocaine, bupivacaine and benzocaine (Schatzmann et al., 1992; Harkins et al., 1996).

Based on the observation that distention of the stomach in the horse provoked a maximum pain sensation, Schatzmann et al. (1992) modified the classical balloon model. A balloon was inserted in the stomach through a nasogastric tube and inflated with oxygen (flow 10L/min). The balloon pressure was used to define PT when the horse showed defined symptoms including restlessness, clear defense reaction and agitation. As this model produces reproducible visceral pain (Schatzmann et al., 1992), it is more economical and practical for evaluation of therapeutics for pain in horses than the classical model.

Based on the well-known effect of a stone in a shoe, Merkens and Schamhardt (1984) developed a reversible lameness model. A special horseshoe was constructed so that a screw could be turned onto the sole surface, resulting in lameness of adjustable severity. After removal of the screw the pain and lameness in horses vanished immediately. Combined with computerized analysis, this model could be used for objective evaluation of degrees of lameness (Merkens and Schamhardt, 1984).

CHAPTER 3
INFLUENCE OF ACUPUNCTURE ON PAIN THRESHOLD IN HORSES

Hypothesis

It is hypothesized that electro-acupuncture induces an increase in pain threshold and changes neuroendocrine responses in horses which are exposed to a noxious heat stimulus.

Subjects and Groups

Twenty-two healthy Thoroughbred horses aged 2 to 13 years were used for this study. Seventeen of these subjects were mares and 5 were geldings. They all were chosen from the Horse Farm at the University of Kentucky. Ten of them were assigned to the study only one time, and 12 of them were assigned twice. For those horses used twice, the washout period was 1 week. The selected horses were required to be free of any pain relief drugs for at least 2 weeks to avoid any confounding influences from these drugs. They were randomly assigned to one of 6 treatment groups including 4 acupuncture treatment groups, a positive and a negative control group (table 3-1 and 3-2):

Acul. Electrical stimulation at acupoints Bai-hui (GV-20a), Duan-Xue (GV-6), left Qiang-feng (SI-9) and left San-yang-lo (SYL) with a frequency of 80-120 HZ.

AcuII. Electrical stimulation at acupoints left SI-9, left SYL, left Qian-chan-wan (QCW) and left Qian-jiu (QJ) with a frequency of 80-120 Hz.

AcuIII. Electrical stimulation at acupoints left SI-9, left SYL, left QCW and left QJ (same as AcuII) with a frequency of 20 Hertz..

AcuIV. Electrical stimulation at acupoints either left SI-9 and left SYL, or left QCW and left QJ, with a frequency of 80-120 Hertz.

Positive control group (PCG). Two milliliters of 0.5 % bupivacaine HCL (10mg/2ml, made in Abbott Lab, North Chicago, IL) was subcutaneously injected into the area of the lateral palmar nerve where it passes lateral (abaxial) to the lateral sesamoid bone (figure 3-1).

Negative control group (NCG). Two milliliters of saline was subcutaneously injected into the same area as described for the PCG group.

For each group, the test was conducted in the barn at the Horse Farm of University of Kentucky. Two hours before the animals were to be tested, they were kept in the quiet stock or barn. They were provided free access to water, but no feed was provided.

One hour before the test, the left and right front pastern were clipped. The clipped areas were blackened with stamp ink (made by Sanford, Beillwood, IL). A catheter was placed in the left jugular vein, filled with heparinized saline and taped to the neck.

Table 3-1 Subjects used in acupuncture trial on pain threshold

Horse ID # or name	sex	age(year)	Group assigned	AM or PM assigned
537	female	13	AcuI; AcuIV	AM PM
542	female	9	AcuII; NCG	AM AM
544	female	5	AcuIII; NCG	AM PM
547	female	13	AcuI; AcuIV	PM PM
552	female	13	AcuI; AcuIV	AM AM
569	female	4	AcuI; AcuIV	PM AM
670	female	5	AcuI	AM
671	female	8	AcuI	PM
684	female	5	AcuII; NCG	AM AM
687	female	9	AcuII; PCG	AM PM
688	female	10	AcuII; PCG	PM AM
695	female	13	AcuIII	PM
696	female	8	AcuIII	PM
697	female	13	AcuII; PCG	PM PM
797	female	5	AcuIII	PM
765	female	2	AcuII	PM
796	female	7	NCG	PM
Brown (202)	male	10	PCG	AM
JD(101)	male	5	AcuII	AM
530	male	9	AcuII; PCG	AM AM
549	male	12	AcuI; NCG	PM PM
795	male	7	AcuI	AM

Table 3-2 Subjects and groups in pain threshold trial

Group	# of horses	# of horses		# of horses (years)			treatment
		male	female	<6	6-9	>9	
AcuI	8	2	6	2	2	4	EA at 4 acupoints with freq of 80-120 Hz
AcuII	8	2	6	3	3	2	EA at 4 acupoints with freq of 80-120 Hz
AcuIII	4	0	4	2	1	1	EA at 4 acupoints with freq of 20 Hz
AcuIV	4	0	4	1	0	4	EA at 2 points with freq of 80-120 Hz
PCG	5	2	3	0	2	3	Bupivacaine injection
NCG	5	1	4	2	2	1	Saline injection

Methods

Pain Threshold Measurement: Hoof Withdrawal Reflex Latency

This measurement is based on Harkins' method (Harkins et al., 1996). The hand-held KHT radiant heat lamp (figure 3-2) was used to elicit the focused radiant light (diameter: 0.4 cm), which was used as a noxious stimulus and was directed onto the pastern to elicit the classic flexion-withdrawal reflex. Hoof withdrawal reflex latency (HWRL) is defined as the time (seconds) between lamp illumination and withdrawal of the hoof. The unfocused light beam (nonpainful light) was also used on the pastern in order to avoid the visual influence from the light. The time could be adjusted by varying the intensity of the heat output of the lamp. The duration of light exposure was limited to 20 seconds for acupuncture and saline treatment groups, and limited to 10 seconds for bupivacaine treatment group to prevent undue damage to the skin.

The HWRL was measured at -15, -5, 0, 15, 30, 60, 90 and 180 minutes for each subject in all 6 groups. The 0 minute time period was the time immediately after completion of any treatment. For the 4 acupuncture treatment groups, the HWRL was also measured at "during" period which is 15 minutes after the beginning of the 30-minute acupuncture stimulation. There was no "during" period in 2 control groups. The HWRL was measured on the front right and left legs at each time period. The HWRL at -15' and -5' time period were considered as baseline: HWRL baseline = (HWRL at -15' + HWRL at -5') ÷ 2.

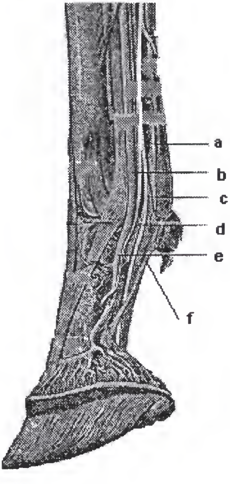


Figure 3-1 Location of nerve block

a: lumbricalis muscle; b: dorsal digital nerve
c: volar digital nerve; d: digital artery;
e: digital vein; f: location of injection

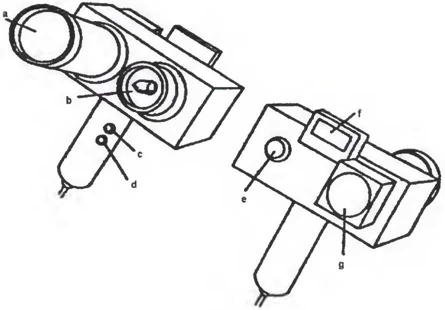


Figure 3-2 Hand held KHT radiant heat lamp

a: Focused light; b: Unfocused light;
c: Unfocused light switch; d: Focused light switch;
e: Reostat; f: Timer; g: Fan

In order to limit the individual variance, the data were transformed to the percent of the baseline (Harkins et al., 1996; Wei, 1990):

% change in HWRL = (HWRL ÷ baseline) × 100.

Acupuncture Procedure

The following acupuncture procedure was based on experience and other research results (Xie, 1994; Yu et al., 1995; Sun, 1986; Klide and Martin, 1989; and Xie and Wang, 1991). A filiform needle (0.30 mm X 75 mm, Suzhou Medical Instrument Factory, Jiangsu, China, figure 3-3) was inserted into each acupoint. An electro-acupuncture Instrument (Model WQ6F, Donghua Electronic Instrument Factory, Beijing, China, figure 3-4) was used to supply electric current. Electrodes were attached to needles that were inserted into the acupoints. Two acupoints were hooked together with electrical wire: GV-20a with GV-6, SI-9 with SYL, and QCW with QJ. The wire was connected to the EA instrument. The electric current was carried through the needle and acupuncture points to the body. Electrical stimulation was conducted for 30 minutes in all 4 acupuncture treatment groups. Different acupuncture groups varied by acupoints and electrical frequency (table 3-2). The location of each acupoint and its manipulating method are described in table 3-3 and figure 3-5.

Blood Chemistry Tests

Blood samples were collected at -15, -5, 0, 15, 30, 60, 90 and 180 minutes for each subject in all 6 groups. The "0" time period was the time immediately after the end of any treatment. There were two sessions each day:

morning and afternoon sessions. The morning session (n=17) started at 0900 hour, and the afternoon session (n=17) started at 1200 hour. Blood samples (20 ml) were collected from jugular vein after clearing the catheter of heparin. Blood was collected into a venipuncture tube containing EDTA as the anticoagulant. The sample tube was immediately inserted in ice, and blood was centrifuged within 30 minutes of collection and stored at -20 ^{0} C until it was assayed. Radioimmunological assay (RIA) was used to measure plasma concentration of β-endorphin (Bossut et al., 1983b), cortisol (Rijinberk et al., 1988) and ACTH (Moore et al., 1979). The β-endorphin kits were purchased from Nichols Institute Diagnostics (San Juan Capistrano, CA). The coat-a-count cortisol kits were purchased from the Diagnostic Products Corporation (Los Angeles, CA). The ACTH kits were from Diagnostic Systems Laboratories, Inc. (Webster, TX). The plasma hormone concentration at -15' and -5' was considered as the baseline: The baseline = (value at -15' + value at -5') ÷ 2.

Design and Analysis of Data

This is a parallel arm design with 2 factors. Data are presented as mean±s.e. Data were analyzed using the general linear models (GLM). The significant level was 0.05 when the Duncan's Multiple Range Test was used to compare data for the different time periods of the same group. The significant level was 0.01 when Least Squares Means were used to compare data for the different treatment groups at each measuring time. The statistical analyses were performed using SAS for Windows (version 6.12).

Table 3-3 Location and manipulation of acupuncture points in this study

Name of acupoint	Location	Manipulating method
Bai-hui (GV-20a)	Between the spinous processes at the lumbosacral junction	Perpendicular insertion for 7.5 cm deep.
Duan-xue (GV-6)	Between the spinous processes of T18 and L1	Perpendicular insertion for 5 cm deep.
Qiang-feng (SI-9)	In the depression formed by the intramuscular groove that separates the long and the lateral heads of m. Triceps brachii just caudal to the deltoideus muscle	Perpendicular insertion for 7.5 cm deep.
San-yang-lo (SYL)	In the intramuscular groove between lateral extensor of the digit and m. Ulnaris lateralis, about 3 cm distal to the proximal head of the radius	Angular insertion for 6 cm deep.
Qian-chan-wan (QCW)	On the caudolateral of the fetlock	Perpendicular insertion for 3 cm deep.
Qian-jiu (QJ)	In the middle of the caudal surface of the hoof, in the depression between the two ends of the hoof cartilage	Angular insertion for 4 cm deep.

Figure 3-3 Filiform needle used in this study

Figure 3-4 Electro-acupuncture Instrument (WQ6F)

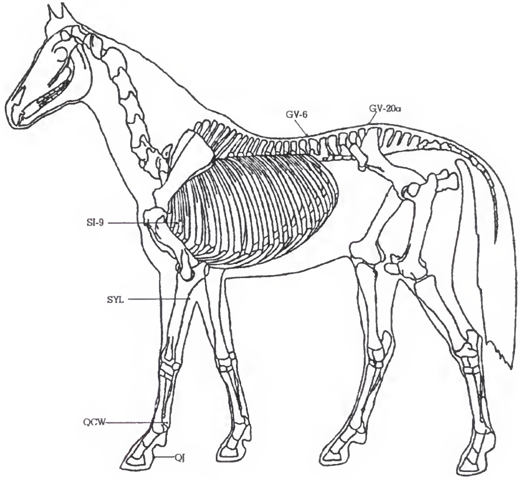


Figure 3-5 Location of acupuncture points used in horses

GV-6: Duan-xue; GV-20a: Bai-hui; QCW: Qian-chan-wan; QJ: Qian-jiu; SI-9: Qiang-feng; SYL: San-yang-lo

Results

Pain Threshold

HWRL in left limb. The data are summarized in table 3-4 and figure 3-6. For the bupivacaine treatment group(PCG), pain threshold increased significantly by 66-86% from 15 minutes up to 120 minutes after treatment ($P<0.001$). For the AcuII group, pain threshold increased by 79% at during, 111% at 0' and 85% at 15' time periods ($P<0.005$). For the AcuIV group, pain threshold increased significantly ($P<0.05$) by 74% at during and 97%at 0 time period. For the groups AcuI, AcuIII and NCG, there was no significant change ($P>0.05$) among different time periods. This result showed that different acupuncture treatments induced different responses to the painful stimulus in horses. The differences in assignment of treatment among the 4 acupuncture groups were either acupoints or frequency of electrical stimulation. The same acupoints were used in AcuII and AcuIII, but different frequencies (80-120Hz for AcuII and 20 Hz for AcuIII) were used. The different results of these two groups indicated that the high frequency of 80-120 Hz may be better for electro-acupuncture analgesic effect.

Even though the same frequency of electrical stimulation (80-120 Hz) was used in the both AcuI and AcuII group, the different acupoints were used in these 2 groups. The different results between these 2 groups indicated that acupuncture analgesic effect depends upon a specificity of acupoints. In other words, different acupoints may result in different physiological changes.

Table 3-4 Effect of acupuncture on % change in HWRL in left limb of horses

Group	# of horse	% change in HWRL (Mean ± s.e.)									P** value
		baseline*	during	0	15'	30'	60'	90'	120'	180'	
AcuI	8	$100\pm0^{a\ 1}$	$122\pm10^{a\ 1}$	$123\pm10^{a\ 1}$	$118\pm11^{a\ 1}$	$96\pm6^{a\ 1}$	$106\pm7^{a\ 1}$	$123\pm12^{a\ 12}$	$110\pm9^{a\ 1}$	$100\pm7^{a\ 1}$	0.1288
AcuII	8	$100\pm0^{a\ 1}$	$179\pm29^{b,c\ 1}$	$211\pm23^{c\ 3}$	$185\pm17^{b,c\ 2}$	$148\pm23^{a,b\ 23}$	$130\pm14^{a,b\ 1}$	$133\pm22^{a,b\ 12}$	$126\pm17^{a,b\ 12}$	$131\pm17^{a,b\ 1}$	0.005
AcuIII	4	$100\pm0^{a,b\ 1}$	$146\pm15^{b\ 1}$	$144\pm16^{b\ 123}$	$118\pm18^{a,b\ 1}$	$113\pm4^{a,b\ 12}$	$136\pm25^{b\ 12}$	$103\pm3^{a,b\ 1}$	$104\pm12^{a,b\ 1}$	$89\pm13^{a\ 1}$	0.0585
AcuIV	4	$100\pm0^{a\ 1}$	$174\pm40^{b,c\ 1}$	$197\pm39^{c\ 23}$	$119\pm15^{a,b\ 1}$	$100\pm11^{a\ 12}$	$107\pm13^{a\ 1}$	$92\pm11^{a\ 1}$	$92\pm14^{a\ 1}$	$89\pm11^{a\ 1}$	0.0080
PCG	5	$100\pm0^{a\ 1}$	-	$122\pm10^{a\ 12}$	$178\pm15^{c\ 2}$	$186\pm19^{c\ 23}$	$186\pm19^{c\ 2}$	$179\pm15^{c\ 2}$	$166\pm13^{b,c\ 2}$	$132\pm9^{a,b\ 1}$	0
NCG	5	$100\pm0^{a\ 1}$	-	$111\pm12^{a\ 1}$	$96\pm4^{a\ 1}$	$109\pm11^{a\ 12}$	$98\pm6^{a\ 1}$	$109\pm15^{a\ 1}$	$105\pm8^{a\ 1}$	$102\pm11^{a\ 1}$	0.9410
P value ***		0	0.0499	0.0020	0.0003	0.0051	0.0027	0.0430	0.0179	0.0825	

* Baseline = (HWRL at -15' + HWRL at -5') ÷ 2; % change in HWRL = (HWRL ÷ baseline) × 100.

**GLM analyses results. Mean values with different alphabetic superscripts are significant ($P<0.05$) within the group by the Duncan's Multiple Range Test.

***Mean values with different numerical superscripts are significantly different ($P<0.01$) between groups by the least squares means comparison.

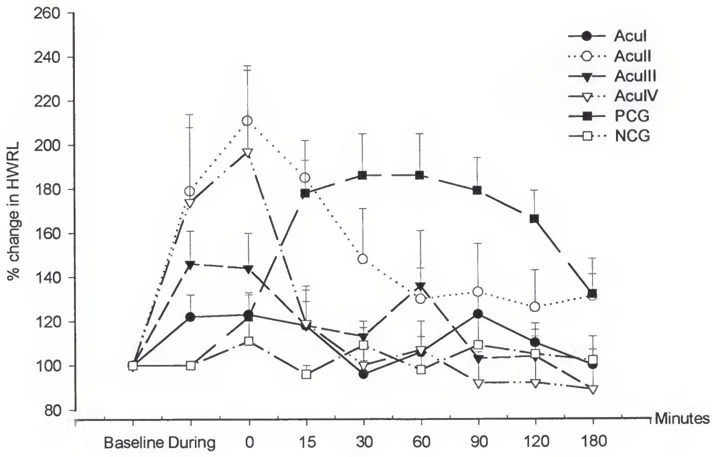


Figure 3-6 Effect of acupuncture on % change in HWRL in left limb of horses

The same frequency (80-120 Hz) was used for both AcuII and AcuIV group. The only difference in these two groups was that only 2 of 4 acupoints used in AcuII group were assigned in AcuIV group. AcuII induced a stronger and longer analgesic effect than AcuIV. This result indicates that more acupoints involved in acupuncture treatment may induce a stronger analgesic effect. This may be related to the accumulation of acupoints.

HWRL in right limb. Pain threshold (HWRL) in the right front leg in horses is listed in table 3-5 and figure 3-7. For PCG, NCG and AcuI groups, pain threshold did not change significantly ($P>0.05$) among the different time periods. However, there was a numerical increase in pain threshold in different time periods in the AcuII and AcuIV. It is of interest that HWRL in left limb (where local acupuncture stimulation took place) did not increase significantly ($P>0.05$) in AcuIII group (table 3-4), while pain threshold in right limb increased significantly by 37% at during time period ($P<0.05$). This may indicate that AcuIII (low frequency of 20 Hz) treatment induced a stronger analgesia in other body areas than the local areas which was close to acupoints.

Plasma Concentration of β-endorphin

Plasma concentration of β-endorphin is summarized in table 3-6 and figure 3-8. Plasma concentration of β-endorphin was highly variable and did not change significantly ($P>0.05$) among different time periods in all groups, even though there was an apparent increase in plasma concentration of β-endorphin at 0', 15' and 30' after treatment in the group AcuII.

Table 3-5 Effect of acupuncture on % change in HWRL in right limb of horses

Group	# of horse	% change in HWRL (mean ± s.e.)									P** value
		baseline*	during	0	15'	30'	60'	90'	120'	180'	
AcuI	8	$100\pm0^{a\ 1}$	$119\pm8^{a\ 1}$	$120\pm8^{a\ 1}$	$108\pm9^{a\ 1}$	$119\pm11^{a\ 1}$	$104\pm5^{a\ 1}$	$116\pm5^{a\ 1}$	$102\pm5^{a\ 1}$	$110\pm8^{a\ 1}$	0.3468
AcuII	8	$100\pm0^{a\ 1}$	$123\pm9^{a,b\ 1}$	$135\pm18^{b\ 1}$	$108\pm7^{a,b\ 1}$	$104\pm9^{a\ 1}$	$105\pm4^{a,b\ 1}$	$101\pm6^{a\ 1}$	$111\pm10^{a,b\ 1}$	$97\pm11^{a\ 1}$	0.1423
AcuIII	4	$100\pm0^{a,b\ 1}$	$137\pm15^{c\ 1}$	$124\pm6^{b,c\ 1}$	$118\pm21^{b,c\ 1}$	$113\pm11^{a,b,c\ 1}$	$99\pm6^{a\ 1}$	$94\pm4^{a,b\ 1}$	$106\pm9^{a,b,c\ 1}$	$81\pm8^{a\ 1}$	0.0398
AcuIV	4	$100\pm0^{a,b\ 1}$	$117\pm16^{a,b\ 1}$	$133\pm10^{b\ 1}$	$95\pm13^{a,b\ 1}$	$88\pm7^{a\ 1}$	$96\pm17^{a,b\ 1}$	$113\pm16^{a,b\ 1}$	$100\pm15^{a,b\ 1}$	$98\pm8^{a,b\ 1}$	0.3251
PCG	5	$100\pm0^{a\ 1}$	-	$112\pm9^{a\ 1}$	$96\pm4^{a\ 1}$	$112\pm8^{a\ 1}$	$112\pm6^{a\ 1}$	$106\pm4^{a\ 1}$	$108\pm7^{a\ 1}$	$97\pm9^{a\ 1}$	0.3487
NCG	5	$100\pm0^{a\ 1}$	-	$108\pm8^{a\ 1}$	$91\pm6^{a\ 1}$	$105\pm9^{a\ 1}$	$112\pm7^{a\ 1}$	$93\pm7^{a\ 1}$	$101\pm12^{a\ 1}$	$85\pm7^{a\ 1}$	0.2242
P value ***		0	0.1442	0.6494	0.5058	0.4680	0.6413	0.1522	0.9616	0.3081	

* Baseline = (HWRL at -15' + HWRL at -5') ÷ 2; % change in HWRL = (HWRL ÷ baseline) × 100.

**GLM analyses results. Mean values with different alphabetic superscripts are significant ($P<0.05$) within the group by the Duncan's Multiple Range Test.

***Mean values with different numerical superscripts are significantly different ($P<0.01$) between groups by the least squares means comparison.

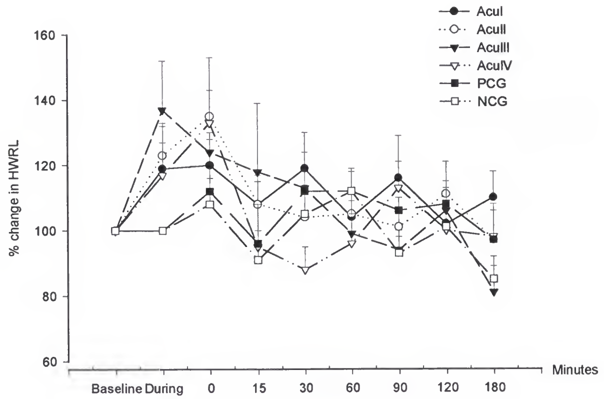


Figure 3-7 Effect of acupuncture on % change in HWRL in right limb of horses

The data of plasma β-endorphin concentration were transformed into percent of baseline as the baseline values varied among each subject (table 3-7 and figure 3-9). Like the original data analyses, there was no significant difference in percent change in plasma concentration of β-endorphin in AcuI, AcuIII, AcuIV, PCG and NCG. But, β-endorphin increased significantly ($P<0.001$) by 211.9% at 0' time period in AcuII group, and continued to increase by 63.3% at 15', 60.3% at 30', and 43.4% at 60' time periods. The plasma β-endorphin level returned to the baseline at 90', and decreased by 24% at 180' time period. These results coincide with the significant increase in the pain threshold (HWRL) in the AcuII group. The endorphin release may be one of the pathways by which the acupuncture stimulation induces analgesic effect.

Even though the HWRL increased significantly at 0' time period in the AcuIV group, the plasma concentration of β-endorphin did not change significantly using this acupuncture treatment. This may be related to different responses due to different acupuncture treatment, or to the other mechanisms by which acupuncture relieves pain.

Plasma concentration of ACTH

Plasma concentration of ACTH is summarized in table 3-8 and illustrated in figure 3-10. Plasma concentration of ACTH in all groups did not change significantly after treatment ($P>0.05$). The data were also transformed into percent change based on the baseline (Appendix table A-1). There were no significant differences

Table 3-6 Influence of acupuncture on plasma concentration of beta-endorphin (pg/ml) in horses

Group	# of horse	Plasma concentration of β-endorphin (pg/ml) Mean ± s.e.								P** value
		baseline*	0'	15'	30'	60'	90'	120'	180'	
AcuI	8	$17.0\pm5.3^{a\,1}$	$19.7\pm7.2^{a\,1}$	$17.8\pm6.1^{a\,1}$	$19.3\pm8.4^{a\,1}$	$14.7\pm4.6^{a\,1}$	$16.5\pm4.3^{a\,1}$	$16.4\pm5.9^{a\,1}$	$21.5\pm9.4^{a\,1}$	0.9976
AcuII	8	$30.1\pm9.9^{a,b\,1}$	$73.7\pm20.5^{b\,1}$	$48.6\pm20.5^{a,b\,1}$	$42.1\pm15.4^{a,b\,1}$	$37.4\pm13.1^{a,b\,1}$	$29.3\pm11.1^{a,b\,1}$	$29.1\pm10.5^{a,b\,1}$	$21.5\pm6.9^{a\,1}$	0.2591
AcuIII	4	$32.2\pm11.0^{a\,1}$	$41.6\pm17.8^{a\,1}$	$66.6\pm41.0^{a\,1}$	$44.6\pm19.1^{a\,1}$	$45.5\pm19.6^{a\,1}$	$31.2\pm10.7^{a\,1}$	$39.8\pm21.0^{a\,1}$	$30.4\pm10.5^{a\,1}$	0.9401
AcuIV	4	$13.8\pm1.0^{a\,1}$	$16.2\pm0.9^{a\,1}$	$14.2\pm0.7^{a\,1}$	$15.9\pm2.5^{a\,1}$	$15.6\pm2.2^{a\,1}$	$16.1\pm1.6^{a\,1}$	$15.5\pm1.4^{a\,1}$	$16.3\pm0.9^{a\,1}$	0.9242
PCG	5	$38.7\pm17.0^{a\,1}$	$39.1\pm21.8^{a\,1}$	$55.0\pm28.6^{a\,1}$	$51.2\pm23.8^{a\,1}$	$36.6\pm13.8^{a\,1}$	$29.2\pm8.4^{a\,1}$	$21.0\pm6.3^{a\,1}$	$32.5\pm14.7^{a\,1}$	0.9133
NCG	5	$22.5\pm9.4^{a\,1}$	$18.5\pm5.8^{a\,1}$	$20.5\pm9.0^{a\,1}$	$18.5\pm6.8^{a\,1}$	$19.1\pm7.7^{a\,1}$	$24.4\pm12.5^{a\,1}$	$23.6\pm11.3^{a\,1}$	$24.5\pm13.3^{a\,1}$	0.9993
P value ***		0.5363	0.0714	0.3829	0.3961	0.2971	0.7669	0.6529	0.9154	

* Baseline = (HWRL at -15' + HWRL at -5') ÷ 2.

**GLM analyses results. Mean values with different alphabetic superscripts are significant (P<0.05) within the group by the Duncan's Multiple Range Test.

***Mean values with different numerical superscripts are significantly different (P<0.01) between groups by the least squares means comparison.

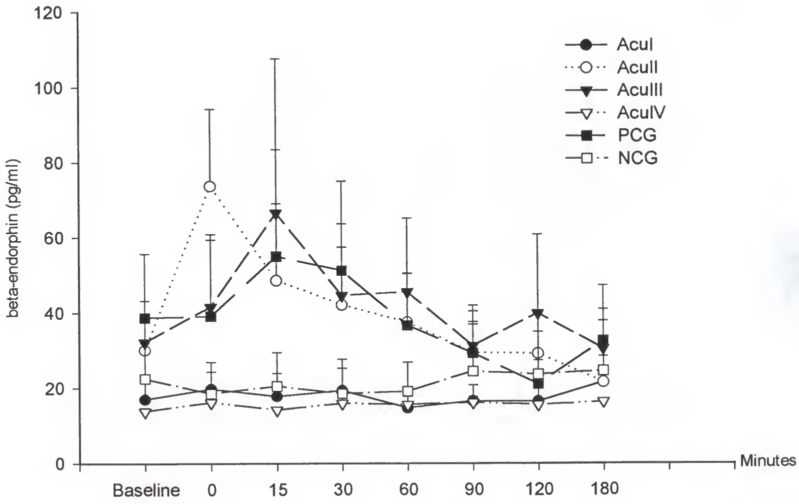


Figure 3-8 Influence of acupuncture on plasma concentration of beta-endorphin (pg/ml) in horses

Table 3-7 % change in plasma concentration of beta-endorphin in horses

Group	# of horse	% change in plasma concentration of β-endorphin (Mean ± s.e.)								P ** value
		baseline*	0'	15'	30'	60'	90'	120'	180'	
AcuI	8	$100.0\pm0^{a\ 1}$	$109.8\pm15^{a\ 1}$	$105.6\pm21^{a\ 1}$	$99.5\pm7^{a\ 1}$	$86.3\pm4^{a\ 1}$	$103.1\pm13^{a\ 1}$	$91.8\pm5^{a\ 1}$	$111.0\pm8^{a\ 1}$	0.7561
AcuII	8	$100.0\pm0^{a,b\ 1}$	$311.9\pm58^{c\ 2}$	$163.3\pm19^{b\ 1}$	$160.3\pm31^{b\ 1}$	$143.4\pm19^{a,b\ 1}$	$96.6\pm8^{a,b\ 1}$	$98.3\pm9^{a,b\ 1}$	$76.0\pm6^{a\ 1}$	0.0001
AcuIII	4	$100.0\pm0^{a\ 1}$	$114.0\pm16^{a\ 1}$	$171.5\pm71.5^{a\ 1}$	$121.5\pm17^{a\ 1}$	$124.0\pm18^{a\ 1}$	$97.5\pm2^{a\ 1}$	$116.8\pm32^{a\ 1}$	$96.8\pm9.0^{a\ 1}$	0.7024
AcuIV	4	$100.0\pm0^{a\ 1}$	$118.5\pm9^{a\ 1}$	$106.0\pm9^{a\ 1}$	$114.8\pm13^{a\ 1}$	$112.0\pm10^{a\ 1}$	$117.5\pm10^{a\ 1}$	$114.3\pm15^{a\ 1}$	$120.8\pm15^{a\ 1}$	0.9023
PCG	5	$100.0\pm0^{a\ 1}$	$94.2\pm15^{a\ 1}$	$117.6\pm18^{a\ 1}$	$117.0\pm13^{a\ 1}$	$106.6\pm20^{a\ 1}$	$110.0\pm31^{a\ 1}$	$79.0\pm14^{a\ 1}$	$90.6\pm12^{a\ 1}$	0.7563
NCG	5	$100.0\pm0^{a\ 1}$	$96.2\pm10^{a\ 1}$	$90.2\pm7^{a\ 1}$	$91.8\pm10^{a\ 1}$	$90.6\pm8^{a\ 1}$	$102.0\pm11^{a\ 1}$	$106.2\pm17^{a\ 1}$	$101.8\pm15^{a\ 1}$	0.9445
P value ***		0	0.0003	0.2154	0.1781	0.0558	0.9406	0.4984	0.0593	

* Baseline = (HWRL at -15' + HWRL at -5') ÷ 2; % change in HWRL = (HWRL ÷ baseline) × 100.

**GLM analyses results. Mean values with different alphabetic superscripts are significant ($P<0.05$) within the group by the Duncan's Multiple Range Test.

***Mean values with different numerical superscripts are significantly different ($P<0.01$) between groups by the least squares means comparison.

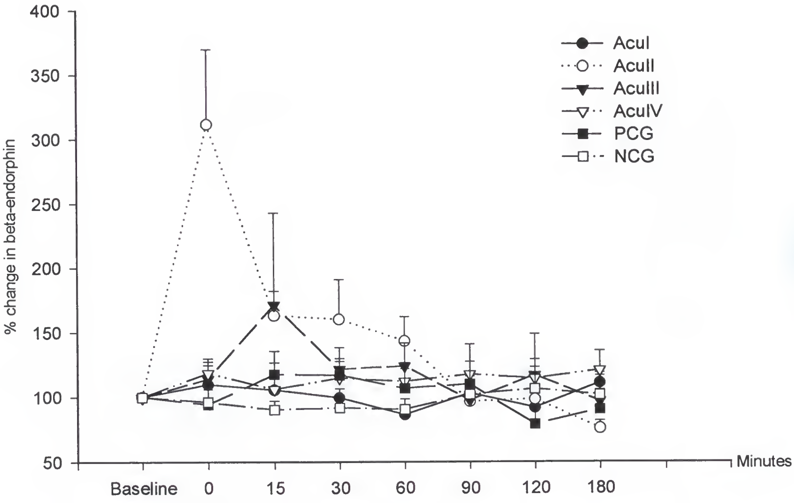


Figure 3-9 Influence of acupuncture on % change in plasma concentration of beta-endorphin in horses

among the different time periods in all groups. This may indicate that the release of ACTH is not the pathway by which acupuncture treatment relieves pain. However, the plasma ACTH level decreased numerically by 25.5% and 23.1% at 30 minutes and 60 minutes after treatment in AcuII group. For PCG group, plasma ACTH decreased by 28.5%, 29.0%, 24.2%, 40.9%, 35.1% and 38.4% respectively at 0, 15, 30, 60, 90 and 120 minutes after treatment. It is unclear if this decrease is related to the local administration of bupivacaine.

Plasma Concentration of Cortisol

Plasma concentration of cortisol is summarized in table 3-9 and figure 3-11. In the saline treatment (NCG) and the 4th acupuncture treatment (AcuIV) plasma concentration of cortisol did not change significantly (P>0.05) among the different time periods. However, there was a decline of plasma cortisol level in the rest of groups. In the 2nd acupuncture treatment (AcuII) group, plasma concentration of cortisol increased numerically at 0' time period, and then decreased at 15 minutes and remained low through out the test. The lowest value occurred at 90 minutes after treatment (P<0.001). The plasma cortisol level in AcuIII group decreased (P<0.001) from 60 up to 180 minutes after treatment. For PCG group, the plasma cortisol level decreased from 90 up to 180 minutes after treatment (P<0.001). For AcuI group, plasma concentration of cortisol appeared to decrease at 90 minutes after treatment (P<0.05). The data on plasma cortisol concentration were transformed into percent change based on the baseline levels (Appendix table A-2). There was no difference (P>0.05) in % change in plasma cortisol levels in AcuIV,

PCG and NCG groups. However, plasma concentration decreased (P<0.005) by 20.4%, 27.4%, 33.1%, 29.6% and 20.7% respectively at 30, 60, 90 120 and 180 minutes after treatment in the AcuI group. For the AcuII group, acupuncture induced at first a significant 23% increase in plasma cortisol level at 0 time period, and then a decrease by 20.2% at 15, 20.9% at 30, 26.1 % at 90 and 21% 180 minutes after treatment (P<0.001). The AcuIII induced a decrease in plasma cortisol concentration from 60 to 180 minutes after treatment (P<0.001).

Table 3-8 Influence of acupuncture on plasma concentration of ACTH (pg/ml) in horses

Group	# of horse	plasma concentration of ACTH (pg/ml) (Mean ± s.e.)								P ** value
		baseline*	0'	15'	30'	60'	90'	120'	180'	
AcuI	8	$93.1\pm9.8^{a\,1}$	$89.0\pm8.8^{a\,1}$	$79.1\pm7.7^{a\,1}$	$83.8\pm8.8^{a\,1}$	$79.0\pm9.3^{a\,1}$	$78.7\pm10.9^{a\,1}$	$76.4\pm11.5^{a\,1}$	$88.2\pm18.5^{a\,1}$	0.9522
AcuII	8	$114.0\pm14.5^{a\,1}$	$95.0\pm13.1^{a\,1}$	$92.7\pm13.1^{a\,1}$	$84.3\pm10.4^{a\,1}$	$85.2\pm10.6^{a\,1}$	$92.3\pm8.6^{a\,1}$	$91.2\pm8.0^{a\,1}$	$91.8\pm12.6^{a\,1}$	0.7317
AcuIII	4	$127.4\pm49.1^{a\,1}$	$69.1\pm12.1^{a\,1}$	$66.6\pm14.2^{a\,1}$	$66.6\pm12.9^{a\,1}$	$75.9\pm9.3^{a\,1}$	$65.9\pm11.9^{a\,1}$	$74.2\pm13.4^{a\,1}$	$69.1\pm20.4^{a\,1}$	0.5083
AcuIV	4	$95.2\pm16.2^{a\,1}$	$91.9\pm11.6^{a\,1}$	$73.0\pm18.9^{a\,1}$	$87.6\pm26.1^{a\,1}$	$95.2\pm18.6^{a\,1}$	$116.4\pm38.3^{a\,1}$	$94.1\pm28.6^{a\,1}$	$88.1\pm11.8^{a\,1}$	0.9577
PCG	5	$94.0\pm18.8^{a\,1}$	$67.0\pm13.7^{a\,1}$	$59.3\pm5.9^{a\,1}$	$67.4\pm11.9^{a\,1}$	$54.4\pm11.2^{a\,1}$	$58.1\pm9.5^{a\,1}$	$55.8\pm9.0^{a\,1}$	$70.8\pm10.1^{a\,1}$	0.3382
NCG	5	$100.2\pm17.2^{a\,1}$	$93.1\pm15.0^{a\,1}$	$86.5\pm10.1^{a\,1}$	$86.6\pm8.6^{a\,1}$	$82.1\pm8.5^{a\,1}$	$79.0\pm7.7^{a\,1}$	$86.3\pm9.6^{a\,1}$	$90.7\pm9.8^{a\,1}$	0.9383
P value ***		0.8215	0.5025	0.3724	0.7741	0.3227	0.1856	0.3830	0.8542	

* Baseline = (HWRL at -15' + HWRL at -5') ÷ 2.

**GLM analyses results. Mean values with different alphabetic superscripts are significant ($P<0.05$) within the group by the Duncan's Multiple Range Test.

***Mean values with different numerical superscripts are significantly different ($P<0.01$) between groups by the least squares means comparison.

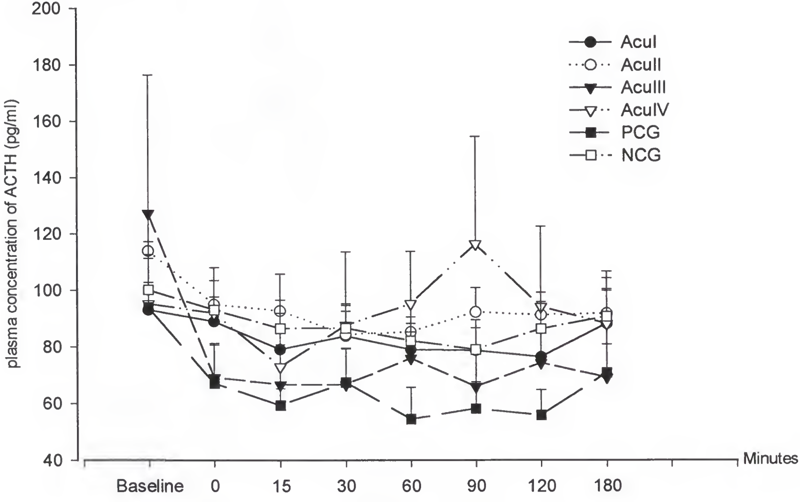


Figure 3-10 Influence of acupuncture on plasma concentration of ACTH (pg/ml) in horses

Table 3-9 Influence of acupuncture on plasma concentration of cortisol (μg/dL) in horses

Group	# of horse	Plasma concentration of Cortisol μg/dL) (Mean ± s.e.)								P **
		baseline*	0'	15'	30'	60'	90'	120'	180'	value
AcuI	8	$104.6\pm7.8^{b\,1}$	$97.7\pm10.7^{a,b\,1}$	$94.1\pm12.6^{a,b\,1}$	$85.1\pm10.8^{a,b\,1}$	$77.6\pm9.9^{a,b\,1}$	$70.7\pm9.2^{a\,1}$	$73.5\pm8.2^{a,b\,1}$	$80.5\pm7.8^{a,b\,1}$	0.1671
AcuII	8	$97.6\pm 7.0^{b,c\,1}$	$120.0\pm11.8^{c\,1}$	$78.5\pm8.6^{a,b\,1}$	$77.3\pm7.4^{a,b\,1}$	$77.7\pm6.5^{a,b\,1}$	69.0 ± 8.7^{a} 1	$79.4\pm7.3^{a,b\,1}$	$74.9\pm4.6^{a,b\,1}$	0.0007
AcuIII	4	$97.7\pm9.8^{d\,1}$	$96.0\pm9.6^{d\,1}$	$88.4\pm5.2^{d\,1}$	$80.6\pm3.9^{c,d\,1}$	$67.0\pm6.2^{b,c\,1}$	$57.6\pm2.1^{a,b\,1}$	$48.7\pm1.1^{a,b\,1}$	$45.3\pm9.4^{a\,1}$	0.0001
AcuIV	4	$97.7\pm9.1^{a\,1}$	$98.0\pm10.3^{a\,1}$	$81.3\pm11.8^{a\,1}$	$82.5\pm10.1^{a\,1}$	$81.9\pm6.9^{a\,1}$	$88.0\pm13.5^{a\,1}$	$95.3\pm5.0^{a\,1}$	$91.3\pm10.0^{a\,1}$	0.8168
PCG	5	$90.3\pm9.2^{c,d\,1}$	$79.2\pm5.6^{bcd\,1}$	$92.9\pm6.5^{d\,1}$	$91.0\pm8.7^{c,d\,1}$	$77.3\pm2.6^{b\,cd\,1}$	$61.2\pm3.2^{a,b\,1}$	$54.9\pm4.3^{a\,1}$	$73.2\pm4.5^{b,c\,1}$	0.0003
NCG	5	$106.6\pm12.0^{a\,1}$	$108.3\pm14.0^{a\,1}$	$99.6\pm9.2^{a\,1}$	$96.1\pm8.6^{a\,1}$	$84.9\pm9.2^{a\,1}$	$79.1\pm11.8^{a\,1}$	$74.8\pm12.7^{a\,1}$	$84.9\pm6.8^{a\,1}$	0.2525
P value ***		0.8407	0.2136	0.6802	0.7399	0.8385	0.3973	0.0179	0.0118	

* Baseline = (HWRL at -15' + HWRL at -5') ÷ 2.

**GLM analyses results. Mean values with different alphabetic superscripts are significant ($P<0.05$) within the group by the Duncan's Multiple Range Test.

***Mean values with different numerical superscripts are significantly different ($P<0.01$) between groups by the least squares means comparison.

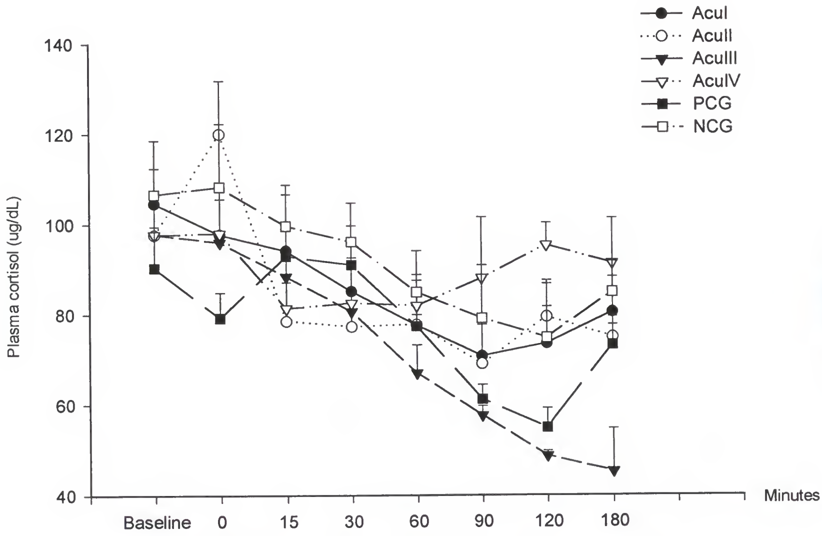


Figure 3-11 Influence of acupuncture on plasma concentration of cortisol (ug/dL) in horses

CHAPTER 4
INFLUENCE OF ACUPUNCTURE ON EXPERIMENTAL LAMENESS IN HORSES

Hypothesis

It is hypothesized that electro-acupuncture will reduce an experimental lameness in horses and elicit neuroendocrinal responses in the animals.

Subjects and Groups

Six thoroughbred horses aged 4 to 8 years old were used in this study (table 4-1). Three were mares, 2 were geldings and 1 was stallion. They were all kept and fed in the field near the College of Veterinary Medicine at the University of Florida.

Table 4-1 Description of subjects used in the trial in detail

trial #	Horse ID #/name	Age (Years)	Sex	Body Weight (Kg)
1	DSG-25, Easy	4	Gelding	450
2	DSG-2, Fuzzy	5	Gelding	580
3	DSS-3, Flag	5	Stallion	585
4	DSM-5, Star	7	Mare	490
5	DSM-10, Ann	8	Mare	530
6	DSM-9, Wish	5	Mare	470

Two days before the start of the experiment each subject was fit with shoes on both front feet. The shoes were designed to allow controlled pressure to be applied to the sole of the foot. The subjects were randomly classified into 3 groups and each horse was assigned all three treatments according to table 4-2. The tests were performed with a washout period of 1 week between each treatment.

Acupuncture treatment (AT). Electro-acupuncture stimulation was conducted on each horse for 45 minutes with frequency of 80-120 Hz.

Positive control (PC). Six milliliters of 0.025% bupivacaine HCL (5mg/2ml) was injected into pastern for nerve blocking.

Sham control (SC). Six milliliters of saline is injected into pastern.

Table 4-2 Trial design of the lameness study

Trial #	Assignment of Treatment (double 3X3 Latin Square)		
	1st	2nd	3rd
1	AT	PC	SC
2	PC	SC	AT
3	SC	AT	PC
4	AT	SC	PC
5	SC	PC	AT
6	PC	AT	SC

AT: acupuncture stimulation; PC: bupivacaine injection; SC: saline injection

At 0800 hours, the test horse was taken to the quiet barn or stocks and a catheter was placed in the left jugular vein, filled with heparinized saline and taped to the neck. At 0900 hours, each horse was brought into the treadmill room for lameness evaluation. At 0930 hours, the screw on the left shoe was tightened until the lameness score was 2 or more. At 1015 hours, electro-acupuncture stimulation was given to the AT group, or pastern injections of bupivacaine in PC group and saline in SC group (figure 3-1). At 1145 hours, the screw on the left shoe was loosened. At 1330 hours, the experiment terminated and the horse returned to the field.

Methods

Pain Model Procedure

The shoeing procedure was based on the Merkens's method (Merkens and Schamhardt, 1984). A M8-nut (ϕ 8mm) was welded to the inner rim of each branch of a shoe between the quarters and the bars, and a round headed screw (2.5 cm length) was screwed into the nut (figure 4-1). Pressure on the intact sole could be developed by further tightening the screw against the sole. The degree of lameness could be varied by turning the screw tighter or untightening, thereby altering the pressure on the sole. The screw on the left shoe was tightened until the horse's lameness score was 2 or more. Most of horses showed lameness when the screw was turned 3 rounds after it touched the sole. Lameness score, stride lengths and treadmill exercise were evaluated on each

subject at pre-shoeing and post-shoeing to determine the baseline of these measurements.

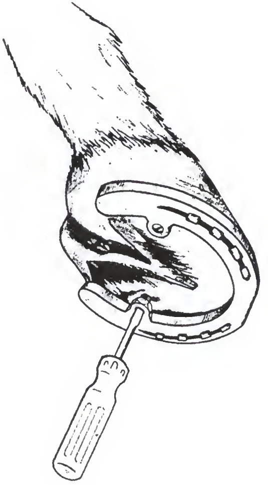


Figure 4-1 Pressure against hoof sole induced by turning the adjustable screw

Lameness Evaluation

Lameness evaluation was based on lameness grading score (Steiss et al., 1989) and stride length (Adams, 1969).

Lameness grading score. Lameness grading score (0 to 3) was designed for evaluating the severity of lameness based on the degree of lameness present (standing, walking and trotting behaviors). All the lameness behaviors were recorded on video-tape for a total of 5 times: before pressure against hoof sole induced by turning the adjustable screw (pre-tightening), after pressure against hoof sole (post-tightening), after the treatment (post-treatment), immediately after the removal of pressure (post-loosening), 95 minutes after the removal of pressure (95' later). Five segments of the lameness behavior of all the horses in each group on the tape were numbered and randomized. The lameness score was the average score of 3 investigators' evaluation. Two of them were blinded. The blinded evaluations were based on the randomized segments of the tape.

0 (no lameness): A horse stands, walks and trots without abnormal gait;

1 (slight lameness): A horse lifts its affected leg incessantly and alternately; no lameness is evident at a walk, but at a trot the horse moves with a short and stilted gait;

2 (Moderate lameness): The horse is willing to move but resists attempts to lift the right leg;

3 (severe lameness): A horse is not willing to move. It only moves when forced.

Stride lengths. The stride lengths were measured from the imprints after the horse walked over a sand pit. The stride length was the average of five continual stride measurements. There are 3 types of stride length: total stride length (TSL), front half stride length (FHSL) and back half stride length (BHSL). The TSL is the distance between the front edges of the imprint of each front leg between successive strides. The TSL of one limb is divided into two parts by the spot where the other limb touches on the ground. The first part is the FHSL, and the last part is the BHSL (figure 4-2). The difference between FHSL and BHSL (DFB) was FHSL minus BHSL: DFB (cm) =FHSL (cm) - BHSL (cm).

Lameness scores and stride length were evaluated 5 times respectively at 0915 hours (before tightening the shoe), 0930 hours (after tightening the shoe), at 1115 hours (after treatment), at 1145 hours (after loosening the shoe) and 1330 hours (95 minutes later after loosening the shoe).

Acupuncture Procedure

Six acupuncture points (3 sets), Bui-hui (GV-20a), Duan-xue (GV-6), the left Qiang-feng (SI-9) and San-yang-luo (SYL), the left Qian-chan-wan (QCW) and Qian-jiu (QJ) were used (table 3-3 and figure 3-5). A tiny needle (0.30 mm X75 mm, Suzhou Medical Instrument Factory, Jiangsu, China, figure 3-3) was inserted into each of the above acupoints. Each set was stimulated by electricity

with 80-120 Hz for 45 minutes using the Electro-acupuncture Instrument (WQ6F, Donghua Electronic Instrument Factory, Beijing, China, figure 3-4).

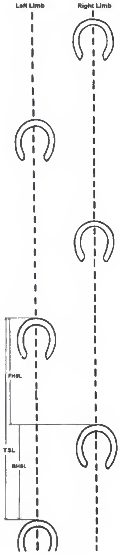


Figure 4-2 The measurement of stride length in horses

TSL: total stride length (cm); FHSL: front half stride length (cm);

BHSL: back half stride length (cm)

Blood Chemistry Tests

Blood samples were collected 14 times respectively at 0800, 0900, 0915, 9030, 0945, 1000, 1015, 1030, 1045, 1100, 1115, 1145, 1200 and 1330 hours for each subject in all 3 groups. Blood samples (20 ml) were collected from jugular vein after clearing the catheter of heparin. Blood was collected into a venipuncture tube containing EDTA as the anticoagulant. The sample tube was immediately inserted in ice, and blood was centrifuged within 30 minutes of collection and stored at -20 ^{0} C until assayed. Radioimmunological assay (RIA) was used to measure plasma concentration of β-endorphin (Bossut et al., 1983b), cortisol (Rijinberk et al., 1988) and ACTH (Moore et al., 1979). The β-endorphin kits were purchased from Nichols Institute Diagnostics (San Juan Capistrano, CA). The coat-a-count cortisol kits and the ACTH kits were from Diagnostic Systems Laboratories, Inc. (Webster, TX).

Design and Analysis of Data

This was a double 3X3 Latin Square design with 2 factors. Data were presented as mean±s.e.. Data were analyzed using the general linear models (GLM). The significance level was 0.05 when the Duncan's Multiple Range Test were used to compare data for the different time periods at the same group. The significance level was 0.01 when Least Squares Means were used to compare data for the different treatment groups at each measuring time. All statistical analysis were performed using SAS for Windows (version 6.12).

Results

Lameness Score

The results are summarized in table 4-3 and figure 4-3. Lameness score increased significantly (P<0.001) in all groups after the shoe was tightened. After saline treatment, lameness score did not change significantly (P>0.05). After bupivacaine injection, lameness score decreased significantly (P<0.001) and returned to baseline level. After acupuncture treatment, lameness score decreased significantly (P<0.001), but did not return to the baseline level.

Table 4-3 Acupuncture on lameness scores in horses

Group	# of horse	Lameness score (Mean ± s.e.)				
		Pre-tightening	Post-tightening	Post-treatment	Post-loosening	95' later
AT	6	0.2 ± 0.2^{a1}	2.7 ± 0.2^{c1}	1.2 ± 0.1^{b2}	0.3 ± 0.2^{a1}	0.3 ± 0.2^{a1}
PC	6	0.3 ± 0.2^{a1}	2.7 ± 0.2^{b1}	0.2 ± 0.1^{a1}	0.1 ± 0.1^{a1}	0.1 ± 0.1^{a1}
SC	6	0.3 ± 0.2^{a1}	2.6 ± 0.1^{b1}	2.5 ± 0.2^{b2}	0.7 ± 0.2^{a1}	0.4 ± 0.1^{a1}

GLM analyses results. Mean values with different alphabetic superscripts are significant (P<0.05) within the group by the Duncan's Multiple Range Test. Mean values with different numerical superscripts are significantly different (P<0.01) between groups by the least squares means comparison.

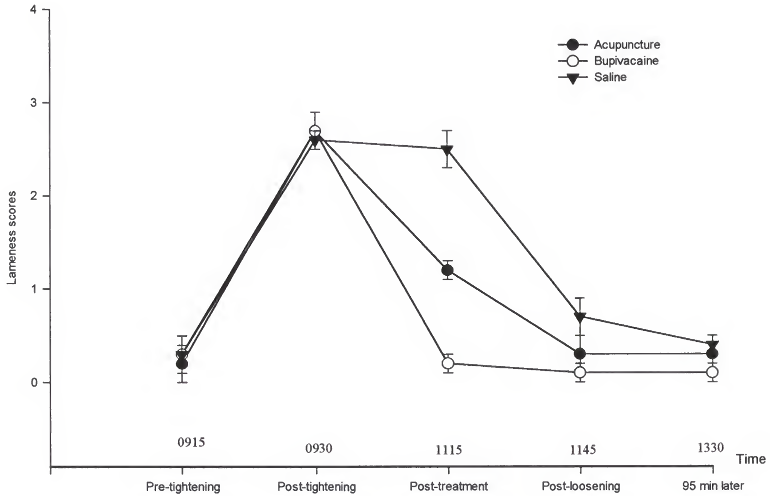


Figure 4-3 Influence of acupuncture on lameness scores in horses

Stride Length

Total stride length. Total stride length (TSL) of the front right or left limb did not change significantly (P>0.05) among different time periods in all groups (table 4-4 and figure 4-4).

Table 4-4 Acupuncture on the total stride length(TSL) * (cm) of the front limb in horses

Group	Limb	Total stride length (cm) (Mean ± s.e.)				
		Pre-tightening	Post-tightening	Post-treatment	Post-loosening	95' later
AT	right	253.5±9.9$^{a\,1}$	234.4 ±8.9$^{a\,1}$	230.1 ±10.8$^{a\,1}$	241.8 ±13.0$^{a\,1}$	247.3 ±11.1$^{a\,1}$
	left	253.2±9.9$^{a\,1}$	234.0±9.2$^{a\,1}$	231.1±11.1$^{a\,1}$	242.3±12.9$^{a\,1}$	247.8±11.1$^{a\,1}$
PC	right	254.0 ±11.9$^{a\,1}$	232.7 ±6.0$^{a\,1}$	245.5 ±9.7$^{a\,1}$	253.4 ±9.1$^{a\,1}$	243.8 ±7.0$^{a\,1}$
	left	254.3±11.8$^{a\,1}$	232.7±5.6$^{a\,1}$	244.7±9.6$^{a\,1}$	253.2±9.0$^{a\,1}$	244.1±6.8$^{a\,1}$
SC	right	243.7 ±9.5$^{a\,1}$	238.4 ±4.0$^{a\,1}$	227.2 ±6.1$^{a\,1}$	249.5 ±6.9$^{a\,1}$	237.3 ±7.8$^{a\,1}$
	left	245.9±9.5$^{a\,1}$	237.9±4.0$^{a\,1}$	227.9±6.1$^{a\,1}$	250.7±7.0$^{a\,1}$	238.0±8.1$^{a\,1}$

* Total stride length (TSL) was the distance between the front edges of the imprint of each front leg between successive strides.

GLM analyses results. Mean values with different alphabetic superscripts are significant (P<0.05) within the group by the Duncan's Multiple Range Test. Mean values with different numerical superscripts are significantly different (P<0.01) between groups by the least squares means comparison.

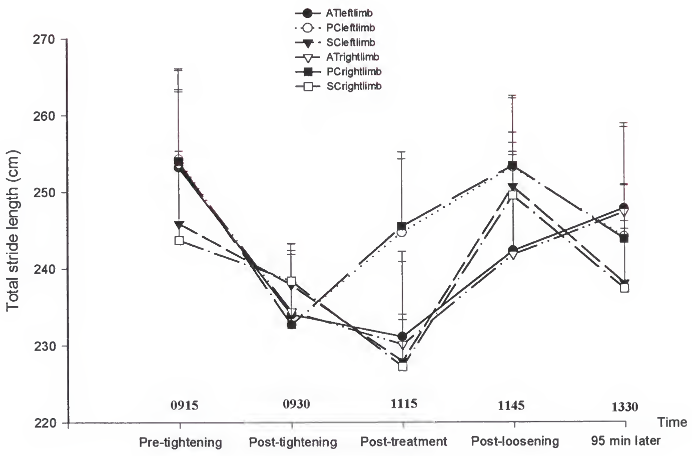


Figure 4-4 Influence of acupuncture on total stride length in horses

<u>The front half stride length and back half stride length of the left front limb</u>. The front half stride length (FHSL) did not significantly (P>0.05) change among different time periods in all groups. The back half stride length (BHSL) did not significantly (P>0.01) change among different time periods in AT and PC groups, however, it decreased (P<0.05) after saline injection in SC group (table 4-5 and figure 4-5).

Table 4-5 Acupuncture on the front half stride length (FHSL) and back half stride length (BHSL) of the left front limb in horses

Group	Half	Stride length (cm) (Mean ± s.e.)				
		Pre-tightening	Post-tightening	Post-treatment	Post-loosening	95' later
AT	FHSL	$126.1 \pm 5.1^{a\,1}$	$122.4 \pm 4.5^{a\,1}$	$117.5 \pm 6.1^{a\,1}$	$120.0 \pm 6.5^{a\,1}$	$123.0 \pm 5.4^{a\,1}$
	BHSL	$127.3 \pm 4.9^{a\,1}$	$111.6 \pm 5.0^{a\,1}$	$113.7 \pm 5.0^{a\,1}$	$122.3 \pm 6.5^{a\,1}$	$124.8 \pm 5.7^{a\,1}$
PC	FHSL	$126.9 \pm 6.0^{a\,1}$	$119.7 \pm 3.7^{a\,1}$	$123.4 \pm 6.0^{a\,1}$	$126.8 \pm 4.4^{a\,1}$	$121.1 \pm 2.7^{a\,1}$
	BHSL	$127.4 \pm 6.0^{a\,1}$	$112.9 \pm 2.6^{a\,1}$	$122.1 \pm 3.9^{a\,1}$	$126.3 \pm 4.8^{a\,1}$	$122.9 \pm 4.4^{a\,1}$
SC	FHSL	$123.1 \pm 4.9^{a\,1}$	$123.2 \pm 2.8^{a\,1}$	$118.4 \pm 3.0^{a\,1}$	$124.5 \pm 4.0^{a\,1}$	$118.7 \pm 3.7^{a\,1}$
	BHSL	$122.8 \pm 4.7^{a\,1}$	$114.7 \pm 2.3^{a,b\,1}$	$109.5 \pm 3.8^{b\,1}$	$126.2 \pm 3.0^{a\,1}$	$119.3 \pm 4.5^{a,b\,1}$

GLM analyses results. Mean values with different alphabetic superscripts are significant (P<0.05) within the group by the Duncan's Multiple Range Test. Mean values with different numerical superscripts are significantly different (P<0.01) between groups by the least squares means comparison.

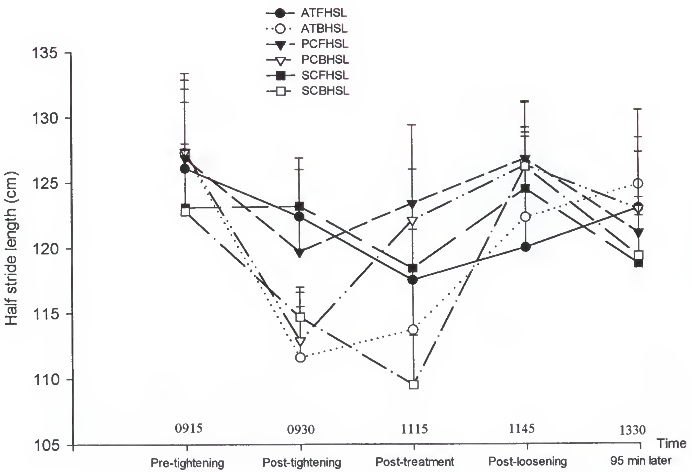


Figure 4-5 Influence of acupuncture on front half stride length (FHSL) and back half stride length (BHSL) in horses

Difference between the front and back half stride length (DFB). Previous to the tightening the shoe (no lameness occurred), the difference between the front and back half stride length (DFB) was -1.2±0.9 cm in AT group, -0.6±1.6 cm in PC group and 0.3±1.3 cm in SC group. When the shoe was tightened (lameness occurred), DFB increased (P<0.01) up to 10.8±2.4 cm in AT group and 8.6 ±3.3 cm in SC group. After acupuncture treatment, DFB decreased (P<0.01) to 3.8 ± 1.3 cm in AT group, while DFB still increased up to 8.9 ±3.2 cm in SC group after saline injection. This result indicates that the DFB may be an objective parameter to assess the lameness in horses. It seems that when a horse was lame, the DFB was over 3.8 cm; and when the DFB was between 0.5 and −2.4 cm, the horse did not show lameness (table 4-6 and figure 4-6).

Table 4-6 Acupuncture on the difference between front and back half stride length (DFB) of left front limb in horses

Group	# of horse	Difference between the front and back half stride length (cm) (Mean ± s.e.)				
		Pre-tightening	Post-tightening	Post-treatment	Post-loosening	95' later
AT	6	-1.2 ± 0.9^{a1}	10.8 ± 2.4^{c1}	3.8 ± 1.3^{b1}	-2.4 ± 0.4^{a1}	-1.8 ± 0.8^{a1}
PC	6	-0.6 ± 1.6^{a1}	6.8 ± 2.9^{a1}	0.5 ± 4.2^{a1}	0.5 ± 2.0^{a1}	-1.8 ± 2.8^{a1}
SC	6	0.3 ± 1.3^{a1}	8.6 ± 3.3^{b1}	8.9 ± 3.2^{b1}	-1.7 ± 1.1^{a1}	-0.6 ± 1.5^{a1}

GLM analyses results. Mean values with different alphabetic superscripts are significant (P<0.05) within the group by the Duncan's Multiple Range Test. Mean values with different numerical superscripts are significantly different (P<0.01) between groups by the least squares means comparison.

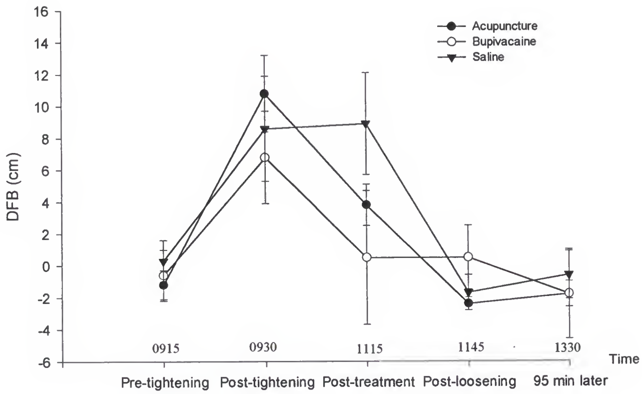


Figure 4-6 Influence of acupuncture on difference between the front and back half stride length (DFB) of the left limb in horses

Plasma Concentration of β-endorphin

For the acupuncture treatment group, plasma concentration of beta-endorphin started to decrease significantly at 0945 hours, but increased significantly ($P<0.001$) after acupuncture treatment, returned to the baseline after termination of acupuncture treatment, and declined to the end of the test (table 4-7 and figure 4-7). For the bupivacaine treatment group, plasma concentration of beta-endorphin started to decrease at 0945 hours, and continued to decline to the end of the test. For the saline treatment group, plasma concentration of beta-endorphin started to decrease at 1000 hours and continued to decline until the termination of experiment.

Plasma Concentration of ACTH

Plasma concentration of ACTH did not change significantly ($P>0.05$) for the different time periods (table 4-8 and figure 4-8).

Plasma Concentration of Cortisol

In the acupuncture group, plasma level of cortisol increased numerically after treatment, but decreased significantly ($P<0.001$) at 1200 and 1330 hours. For both the bupivacaine and saline groups, plasma concentration of cortisol did not change significantly ($P>0.05$) among different time periods (table 4-9 and figure 4-9).

Table 4-7 Acupuncture on plasma concentration of beta-endorphin (pg/ml) in horses

Time hour	Treatment	Plasma concentration of beta-endorphin (Mean ± s.e.)		
		AT (n=6)	PC (=6)	SC (n=6)
800		$50.02 \pm 2.8^{c,d\ 1}$	$49.62 \pm 4.7^{a\ 1}$	$52.91 \pm 17.8^{a\ 1}$
900		$51.73 \pm 2.6^{c,d\ 1}$	$49.32 \pm 6.1^{a\ 1}$	$53.52 \pm 24.2^{a\ 1}$
915		$50.21 \pm 2.3^{c,d\ 1}$	$46.69 \pm 4.4^{a,b\ 1}$	$45.93 \pm 19.4^{a,b\ 1}$
930	Tightening shoe	$44.28 \pm 2.5^{d,e\ 1}$	$44.2 \pm 8.8^{a,b,c\ 1}$	$46.63 \pm 22.0^{a.b\ 1}$
945		$38.76 \pm 4.1^{e\ 1}$	$40.24 \pm 9.1^{b.c.d\ 1}$	$43.36 \pm 17.3^{a,b\ 1}$
1000		$39.19 \pm 2.8^{e\ 1}$	$40.54 \pm 8.9^{b,c,d\ 1}$	$31.51 \pm 18.3^{b,c\ 1}$
1115	AT, PC,or SC	$45.68 \pm 2.8^{d,e\ 1}$	$38.58 \pm 5.0^{b,c,d\ 12}$	$31.24 \pm 14.3^{b,c\ 23}$
1030		$63.44 \pm 4.1^{b\ 1}$	$36.75 \pm 3.6^{c,d\ 2}$	$24.13 \pm 11.5^{c,d\ 2}$
1045		$88.36 \pm 2.1^{a\ 1}$	$32.44 \pm 6.7^{d,e\ 2}$	$23.42 \pm 11.0^{c,d\ 2}$
1100	AT ended	$92.38 \pm 3.6^{a\ 1}$	$27.14 \pm 10.5^{e\ 2}$	$20.16 \pm 6.7^{c,d\ 2}$
1115		$59.39 \pm 3.9^{b,c\ 1}$	$25.05 \pm 7.3^{e,f\ 2}$	$20.98 \pm 12.2^{c,d\ 2}$
1145	Loosening shoe	$49.49 \pm 4.9^{c,d\ 1}$	$17.75 \pm 6.1^{f,g\ 2}$	$11.92 \pm 5.3^{c,d\ 2}$
1200		$37.27 \pm 1.7^{e\ 1}$	$11.39 \pm 2.6^{g\ 2}$	$8.68 \pm 2.0^{d\ 2}$
1330		24.40 ± 2.2^{f}	10.29 ± 2.3^{g}	7.31 ± 2.9^{d}

GLM analyses results. Mean values with different alphabetic superscripts are significant ($P<0.05$) within the group by the Duncan's Multiple Range Test. Mean values with different numerical superscripts are significantly different ($P<0.01$) between groups by the least squares means comparison.

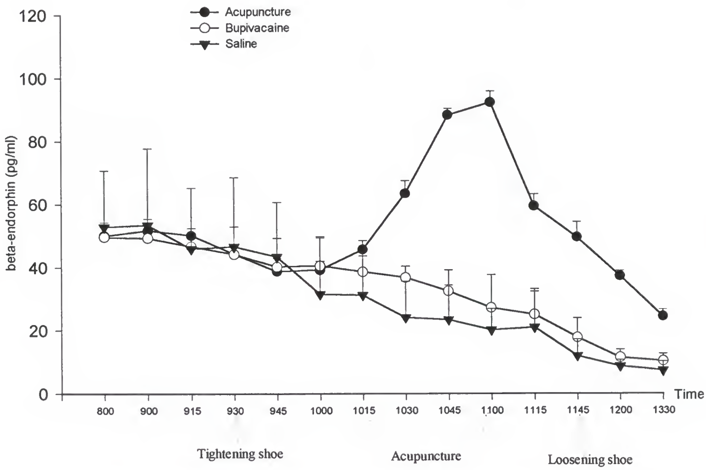


Figure 4-7 Influence of acupunture on plasma concentration of beta-endorphin (pg/ml) in horses

Table 4-8 Acupuncture on plasma concentration of ACTH (pg/ml) in horses

Time period	Treatment	Plasma concentration of ACTH (pg/ml) (Mean ± s.e.)		
		AT (n=6)	PC (=6)	SC (n=6)
800		84.88 ± 20.3 $^{a\ 1}$	96.11± 5.4 $^{a\ 1}$	97.13± 15.2 $^{a\ 1}$
900		77.19 ± 15.3 $^{a\ 1}$	87.24 ± 6.6 $^{a\ 1}$	86.05± 9.7 $^{a\ 1}$
915		69.28 ± 16.1 $^{a\ 1}$	92.33± 4.9 $^{a\ 1}$	86.34 ± 12.5 $^{a\ 1}$
930	Tightening shoe	70.51± 15.4 $^{a\ 1}$	86.37± 7.9 $^{a\ 1}$	85.73± 11.6 $^{a\ 1}$
945		74.75 ± 18.8 $^{a\ 1}$	84.24 ± 6.3 $^{a\ 1}$	81.98± 11.0 $^{a\ 1}$
1000		74.25 ± 14.7 $^{a\ 1}$	87.56 ± 7.8 $^{a\ 1}$	86.93± 14.4 $^{a\ 1}$
1115	AT, PC,SC	69.03± 14.7 $^{a\ 1}$	92.91 ± 9.8 $^{a\ 1}$	82.50± 13.8 $^{a\ 1}$
1030		83.79 ± 20.6 $^{a\ 1}$	91.17 ± 10.2 $^{a\ 1}$	84.42± 15.6 $^{a\ 1}$
1045		69.87 ± 13.6 $^{a\ 1}$	89.67 ± 10.5 $^{a\ 1}$	96.04± 14.9 $^{a\ 1}$
1100	AT ended	69.71 ± 14.4 $^{a\ 1}$	91.79 ± 10.6 $^{a\ 1}$	101.27 ± 17.5 $^{a\ 1}$
1115		71.79 ± 14.9 $^{a\ 1}$	93.05 ± 11.1 $^{a\ 1}$	101.59± 14.1 $^{a\ 1}$
1145	Loosening shoe	64.24± 14.0 $^{a\ 1}$	86.36 ± 5.8 $^{a\ 1}$	92.51± 11.5 $^{a\ 1}$
1200		67.31± 12.8 $^{a\ 1}$	98.87±9.0 $^{a\ 1}$	86.14±11.7 $^{a\ 1}$
1330		71.88± 15.0 $^{a\ 1}$	104.5 ± 13.5 $^{a\ 1}$	82.94± 8.8 $^{a\ 1}$

GLM analyses results. Mean values with different alphabetic superscripts are significant (P<0.05) within the group by the Duncan's Multiple Range Test. Mean values with different numerical superscripts are significantly different (P<0.01) between groups by the least squares means comparison.

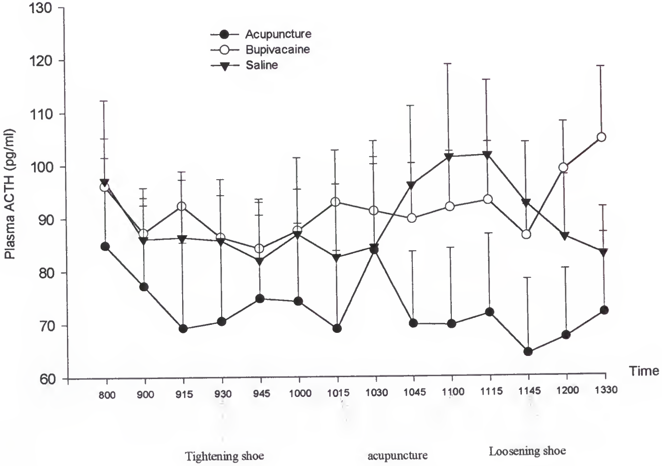


Figure 4-8 Influence of acupunture on plasma concentration of ACTH (pg/ml) in horses

Table 4-9 Acupuncture on plasma concentration of cortisol (μg/dL) in horses

Time hour	Treatment	plasma concentration of cortisol (μg/dL) (Mean±s.e.)		
		AT (n=6)	PC (n=6)	SC (n=6)
800		21.48 ± 1.3 $^{a,b,c,d\ 1}$	20.90± 4.3 $^{a\ 1}$	19.41± 3.6 $^{a\ 1}$
900		22.44 ± 2.7 $^{a,b,c\ 1}$	14.0 ± 3.1 $^{a\ 1}$	19.89± 3.8 $^{a\ 1}$
915		26.40± 2.3 $^{a,b\ 1}$	17.86 ±3.4 $^{a\ 1}$	20.44 ±3.7 $^{a\ 1}$
930	Tightening shoe	21.76 ± 1.4 $^{a,b,c,d\ 1}$	16.77 ± 3.5 $^{a\ 1}$	16.87± 3.2 $^{a\ 1}$
945		20.33 ± 1.2 $^{c,b,d\ 1}$	16.70± 2.9 $^{a\ 1}$	14.33±3.2 $^{a\ 1}$
1000		22.87 ± 1.9 $^{a,b,c\ 1}$	17.32 ± 1.8 $^{a\ 1}$	18.09± 4.2 $^{a\ 1}$
1115	AT, PC,SC	26.14± 2.1 $^{a,b\ 1}$	21.97 ± 2.1$^{a\ 1}$	22.64±5.1 $^{a\ 1}$
1030		25.97 ± 2.4 $^{a,b\ 1}$	22.62 ± 2.0 $^{a\ 1}$	22.1 ± 4.0 $^{a\ 1}$
1045		29.11 ± 3.2 $^{a\ 1}$	20.03 ± 2.4 $^{a\ 1}$	23.91 ± 3.6 $^{a\ 1}$
1100	AT ended	26.71± 2.0 $^{a,b\ 1}$	18.57± 2.5 $^{a\ 1}$	25.49 ± 3.7 $^{a\ 1}$
1115		25.70± 4.3 $^{a,b,c\ 1}$	19.35 ± 3.0 $^{a\ 1}$	26.76± 3.2 $^{a\ 1}$
1145	Loosening shoe	17.83 ± 2.4 $^{c,d,e\ 1}$	13.6± 3.2 $^{a\ 1}$	19.94 ±2.3 $^{a\ 1}$
1200		12.72 ± 2.6 $^{e\ 1}$	15.93 ±3.1 $^{a\ 1}$	17.73 ± 2.6 $^{a\ 1}$
1330		14.05 ± 2.8 $^{e\ 1}$	17.49 ±2.9 $^{a\ 1}$	12.81± 3.8$^{a\ 1}$

GLM analyses results. Mean values with different alphabetic superscripts are significant (P<0.05) within the group by the Duncan's Multiple Range Test. Mean values with different numerical superscripts are significantly different (P<0.01) between groups by the least squares means comparison.

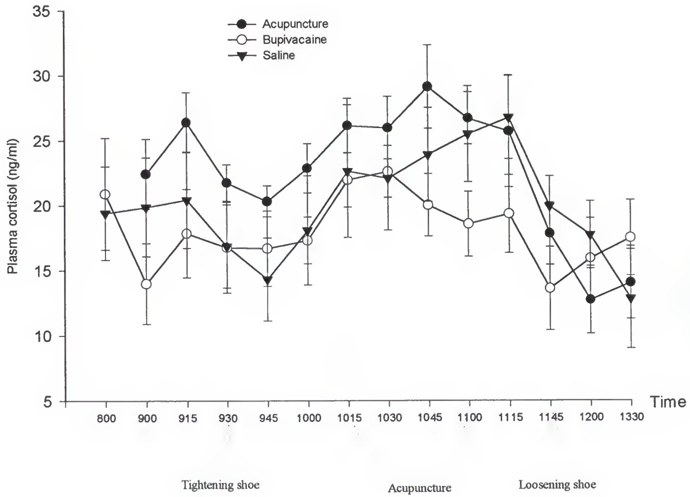


Figure 4-9 Influence of acupuncture on plasma concentration of cortisol (ng/ml) in horses

CHAPTER 5
DISCUSSION

Pain Threshold

Pain threshold (PT) has been used to evaluate acupuncture analgesic effect in horses (Bossut et al., 1984), cattle (Zhang et al., 1981), sheep (Bossut et al., 1986), pigs (Zhu, 1992), dogs (Wu and Huang, 1988), and rabbits (Yu and Zhong, 1985). Heat, pinprick and pinch were the most commonly used noxious stimuli for measurement of PT in horses (Bossut et al., 1984; and Harkins et al., 1996). An increase in PT was interpreted as analgesia induced by treatment. In this study, the focused radiant light beam was used to produce noxious heat which was directed onto the pastern to elicit the classic flexion-withdrawal reflex. The hoof withdrawal reflex latency (HWRL) was used to measure PT. PT determination was conducted in the both front limbs. Four different acupuncture treatments (different acupoints and frequency of electrical stimulation) were used.

On the basis of the original data analysis of the left front limb, the AcuII group (80-120 Hz of frequency and 4 local acupoints including left Qiang-feng, San-yang-luo, Qian-chan-wan and Qian-jiu) induced a significant increase in PT of the left front limb ($P<0.001$). This result coincides with other data and the

general acceptance of acupuncture analgesic effect (Bossut et al., 1984 and 1986; Fleming, 1994; and Richardson et al., 1986). The acupoints Qiang-feng and San-yang-luo were generally used for lameness of the front limb and also considered as a pair acupoint for analgesia for surgery in the Traditional Chinese Veterinary Medicine (TCVM)'s perspective (Yu, 1984 and Xie, 1994). Electro-acupuncture (EA) stimulation in Qiang-feng and San-yang-luo induced an increase in PT across the 5 trunk areas in horses ($P<0.05$) (Bossut et al., 1984). EA stimulation in Qiang-feng and San-yang-luo induced an analgesia strong enough to perform laparotomy in a goat (Liu, 1987). CO_2 Laser stimulation in Qiang-feng and San-yang-luo induced a sufficient acupuncture analgesia for standing laparotomies to be performed in 50 ruminants (including 12 yellow cattle, 8 water buffalo and 30 goats) (Chen et al., 1980a).

In classical TCVM's view, Qian-chan-wan and Qian-jiu are the most commonly used hemo-acupuncture points for acute lameness of the front limb (Yu, 1984 and Xie,1994). No reports have been found in the area in which these two hemo-acupoints are stimulated by electric current. As hemo-acupuncture therapy is not well accepted in the Western countries including the USA, EA or conventional acupuncture stimulation can be used on classical hemo-acupoints. EA at Qian-chan-wan and Qian-jiu has been used successfully for treatment of lameness in 4 Thoroughbred horses (author's unpublished data).

In the AcuIII group, the same 4 acupoints as used in group AcuII were used but with 20 Hz of frequency no change ($P>0.05$) in PT of the left front limb

was detected (table 3-4) . The results indicate that high frequency (80-120 Hz) electrical stimulation at acupoints may induce a stronger local analgesic effect than a low frequency (20 Hz). This result did not coincide with Chen and Wang's findings that low frequency (2-3 Hz) electrical stimulation had a stronger and longer analgesic effect in cattle and sheep than high frequency (50-300 Hz) (Chen and Wang, 1983). On the basis of clinical analysis of 575 cases associated with acupuncture analgesia in cattle and sheep, they found that the EA with high frequency induced the greatest increase in PT within 5-15 minutes, but this acupuncture analgesia declined very quickly after the termination of EA stimulation and returned to the baseline within 1.5 hours. The EA with low frequency induced the highest peak 30 minutes after the beginning of stimulation and this acupuncture analgesia could last up to 2-4 hours after the termination of acupuncture stimulation (Chen and Wang, 1983). The different results may be related to the different species. The location of PT test could also be a factor as the trunk (especially in flank) is generally considered a better analgesia area than the limb (especially in pastern and hoof) (Zhu, 1992; and Yu, 1984; Bossut et al., 1984). The flank was used for locus of noxious stimulation in Chen and Wang's research (Chen and Wang, 1983) while the pastern was used the locus of noxious stimulation in this study.

In the AcuI group, 2 acupoints in the back, Bai-hui and Duan-xue,, and 2 acupoints in the left front limb, Qiang-feng and San-yang-luo were used with a frequency of 80-120 Hz of electrical stimulation. This acupuncture treatment did

not change the PT in the left front limb (P>0.05). Like the acupoint Qiang-feng and San-yang-luo, Bai-hui and Duan-xue are generally considered as a pair acupoint for analgesia for abdominal surgeries in TCVM's perspective (Yu, 1984 and Xie, 1994). Failure to induce analgesia using this acupuncture treatment may be related to locus of noxious stimulation as EA in Bai-hui and Duan-xue or in Qiang-feng and San-yang-luo induced a strong analgesia in the flank, back, lumbar areas and abdomen but not in limbs and heads in sheep, goats and pigs (Zhu, 1992). Zhou (1984) also found that acupuncture analgesia was sufficient for 10 abdominal surgeries, but not for 4 limb surgeries in horses.

In the AcuIV group, either 2 acupoints Qiang-feng and San-yang-luo, or Qian-chan-wan and Qian-jiu were used with 80-120 Hz of frequency of electrical stimulation. This acupuncture treatment induced a numeric increase in PT (P>0.05). The combination of these 4 acupoints in the AcuII group induced a significant increase in PT. This indicates that acupoints may potentiate each other. This result coincides with clinical application of 4-6 acupoints for each treatment (Yu, 1984 and Xie, 1994). Bossut et al. (1984) found that acupuncture analgesic efficacy varied between sexes in horses. Analgesia was induced equally well in both castrated males and intact females by the EA stimulation in Yao-pang 3 points, Bai-hui and Ba-shan, however, EA stimulation in Bai-hui and Ba-shan caused a significant analgesia in females only (Bossut et al., 1984). No difference in acupuncture analgesic effect was found in our study, however, only 5 males (7 times) but 17 mares (27 times) were used in this study. The bias of

sex could have contributed to the different results. However, 3 females and 3 males were used in our second project (sole pressure project). No significant difference between mares and male horses was found in EA effectiveness on lameness scores and neuroendocrine responses. The precise role of sex in EA effectiveness requires further clarification.

In order to limit the individual variance, the data were transformed to the percent of the baseline (Harkins et al., 1996; Wei, 1990). The HWRL baseline varied from 2.2 to 7.8 seconds in this study, thus the data were transformed to the percent of the baseline. In the AcuII group, PT of the left limb increased by 79% at during, 111% at 0' and 85% at 15' time periods, and continued to maintain the numeric increase up to 180 minutes after the termination of acupuncture treatment. In the AcuIV group, PT in the left limb increased by 74% at during and 97% at 0 time periods, and then returned to the baseline level at 15 minutes after the termination of acupuncture stimulation. The only difference between AcuII and AcuIV groups was the acupoints. As mentioned above, only 2 acupoints (1 pair) of the 4 acupoints (2 pairs) used in AcuII were selected for AcuIV. The better acupuncture analgesic effect from the AcuII group indicates that some acupoints could potentiate the other acupoints. Even though the PT was consistent with the increase up to 180 minutes after the termination of acupuncture treatment in the AcuII group, the PT in the AcuIV group started to decline by 8% at 90' and 120' and 11% at 180' time periods. The reasons for this declination remain to be determined.

In the AcuIII group, PT in the left limb increased inconsistently by 44-46% from during to 0 time period, 13-18% from 15 to 30', by 36% at 60' , by 3-4% from 90 to 120' time period and then dropped by 11% at 180' time period, but this change in PT was not significant ($P>0.05$). The reasons for this inconsistent change in PT in this acupuncture treatment remain unclear. The number of animals used in this group was only 4 and may be related to this result.

Acupuncture treatments, bupivacaine and saline injection were conducted only in the left limb. The positive control bupivacaine injection induced a significant increase in PT only in the left limb ($P<0.05$) but not in the right limb. Thus, the contralateral, or the non-treated limb was considered as a controlled limb. In this study, the left limb is the treated limb while the right limb is the controlled limb. For the AcuII group, PT in the controlled limb increased by 23% at during and 35% at 0 time period without significance ($P>0.05$). However, when significant level α was set as 0.15, the 35% increase at 0 time period was significant. This result indicates that the acupuncture treatment may induce a whole body analgesia, rather than a local analgesia.

Even though the 3rd acupuncture treatment (low frequency of 20 Hz) did not induce a significant increase in PT of the treated limb, PT in the controlled limb increased significantly by 37% at during time period, and continued to increase by 24% at 0', 18% at 15' and 13% at 30' time periods. This result indicates that low frequency EA stimulation induced a stronger analgesic effect in other body areas than the local areas close to acupoints. It suggests that in

clinical practice the acupoints close to the painful areas should be stimulated with high frequency EA while the acupoints far from the painful areas should be stimulated with low frequency EA. Janssens et al. (1988) suggested that segmental analgesia may be better when high frequency (100 to 1000 Hz) and low intensity stimulation is used, while low frequency (2 to 10 Hz) stimulation causes a more typical generalized analgesia.

Lameness Evaluation

Lameness Score

The results show that EA can decrease the lameness score and reduce the degree of lameness significantly. This coincides with the numerous clinical studies in that acupuncture treatment effectively resolved lameness due to joint contusion, muscular atrophy, chronic back pain, rheumatic pain and laminitis in horses (Yang, 1984; Su, 1982; Xie et al., 1996; Liang, 1982 and Wang, 1990). However, compared with the fact that local anesthetic bupivacaine completely resolved lameness and lameness score returned to the baseline, EA only partially resolved lameness and lameness score decreased by 56% from 2.7 to 1.2. In other words, the EA can partially relieve the pain due to the mechanical pressure induced by tightening the screw against the sole. It indicates that mechanism of acupuncture analgesic effect is not, at least not totally, nerve blocking like local anesthetic. However, acupuncture effect is also related to nerve systems. Acupuncture stimulation on Zu-san-li in the hindlimb induced a decrease in renal sympathetic nerve activity (RNA) as well as mean arterial

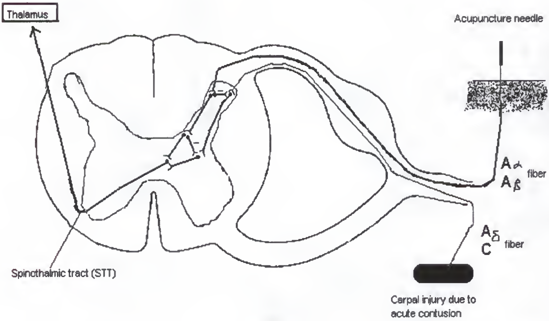
blood pressure (MAP), while transection of sciatic and femoral nerves completely abolished the responses of RNA and MAP (Ohsawa et al., 1995). A sciatic nerve cut abolished the acupuncture analgesic effect in the pain-producing muscle of guinea pigs (Takeshige and Sato, 1996). These results indicate that acupuncture effect may be via the afferent nerve fibers.

Peripheral nerve fibers can be divided into 2 categories: myelinated and unmyelinated (Katz and Ferrante, 1993). There are 5 myelinated fibers: Aα, Aβ, Aγ, Aδ and B, and the unmyelinated fiber referred to as the C fiber (see table 5-1). The primary sensory afferent fiber concerned with nociception is termed the nociceptor. Nociceptive afferents generally fall into the myelinated Aδ fiber and the unmyelinated C fiber, which are called nociceptive afferents. On the other hand, Aα, Aβ, Aγ and B are called nonnociceptive afferents (Katz and Ferrante, 1993). The general principle is that nociceptive afferents have a higher threshold to stimulation than nonnociceptive afferents. Aδ fibers respond to thermal and mechanical stimuli (e.g. pressure) (Adriaensen et al., 1983), while C fibers respond to noxious mechanical, thermal and chemical stimuli and thus are called C-polymodal nociceptors (C-PMNs).

The local anesthetics block the intensive prolonged pain and the secondary prolonged dull sensation by preferentially and directly blocking the C-PMNs. Low-intensity electric stimulation preferentially activates the largest fibers such as Aα and Aβ (Collins et al., 1960). As EA is a type of low-intensity electric stimulation (Yu, 1984) and most of acupoints are motor points (Kendall, 1989),

the author suggests that Aα and Aβ get involved in transduction of EA signals. On the basis of fact that 26 out of 30 horses in this study did present restlessness and discomfort when the needle was inserted into an acupoint, the author also suggests that EA may also stimulate the myelinated Aδ and unmyelinated C fibers. Kendall (1989) also considered that acupuncture was mediated by afferents Aδ and C fibers. In summary, EA stimulates the afferent fibers Aα, Aβ, Aδ and C. However, the large Aα and Aβ fibers may be dominant during the transduction of EA stimulation, because: 1) nociceptive afferents Aδ and C have a higher threshold to stimulation than nonnociceptive afferents Aα and Aβ; 2) EA is a type of low intensity stimulation; and 3) the horse remains stable and does not present any noticeable evidence of pain during EA stimulation (Bossut et al., 1984). Painful signals could be influenced and partially or completely blocked by EA stimulation before they reach the dorsal horn of spinal cord (Snader, 1993). For example, EA stimulation for treatment of carpal injury due to acute contusion (See figure 5-1).

The painful signals from carpal joints reached the dorsal horn of the spinal cord via the nociceptive afferent Aδ and C fibers. During the same time, EA signals from acupoints were transmitted to the dorsal horn dominantly by Aα and Aβ, and also by afferents Aδ and C. When painful signals and EA signals faced and influenced each other in the dorsal horn, there were 3 possible outcomes: 1) they did not influence each other and nothing changed; 2) EA signals potentiated painful signals, resulting in more pain; 3) EA signals inhibited



Pain signals were transducted from carpal joints to the dorsal horn of spinal cord via the small afferent Aδ and C fibers. During the EA treatment, EA signals reached the dorsal horn via large Aα and Aβ at the same time. EA signals may inhibit the painful signals.

Figure 5-1 Transduction and transmission of EA and painful signals

painful signals, resulting in less pain or no pain. As Aα and Aβ have a larger diameter and higher conduction velocity, more and faster EA signals reached the dorsal horn than painful signals in the given time. When more EA signals take up "the places" in the dorsal horn first, painful signals have fewer chances to take up "the places" (See figure 5-1). Thus, the 3rd outcome could more likely occur. In other words, EA may induce a segmental spinal inhibition of nociceptive inputs and result in an immediate, short-lasting, segmental and non-opioid analgesia (Ernst and Lee, 1987).

Stride Length

A stride length was defined as the distance between the front edges of the imprint of each front leg between the successive strides. One stride length consists of the front and back half parts. The difference between the front and back half was called the DFB in this study. The front half stride length is similar to Adams' anterior phase of stride and the back half stride length to Adam's posterior phase of the stride (Adams, 1969). The results show that the total stride length of the front right or the left limb did not change significantly among different time periods for all treatment (including saline control) groups. This is because the length of the stride must be the same as the opposite limb to keep the balance even when the horse is lame (Adams, 1969).

Table 5-1 Classification of peripheral nerve fibers

Fiber group	Innervation	Mean diameter (μm)	Mean conduction velocity (m/sec)
Myelinated			
Aα	Primary muscle spindle motor to skeletal muscles	15	100
Aβ	Cutaneous touch and pressure afferents	8	50
Aγ	Motor to muscle spindle	6	20
Aδ	Mechanoreceptors, nociceptors	<3	15
B	Sympathetic preganglionic	3	7
unmyelinated C	Mechanoreceptors, nociceptors, sympathetic postganglionic	1	1

The results show clearly that the DFB increased significantly when the horse was lame. The bigger the DFB was, the more lame the horse. When the screw was tightened against the sole, the horse showed supporting leg lameness, and consequently the back half stride length (BHSL) or posterior phase was shortened. This coincides with the general principle that the lameness in the hoof should cause shorter posterior phase of the stride. When the BHSL is shortened, there must be a compensatory lengthening of the front half stride length (FHSL) or anterior phase, resulting in the increase of the DFB.

When the screw was tightened enough against the sole to induce obvious lameness (lameness scores ≥ 2.0), the DFB increased up to 10.8 ± 2.4 cm in the acupuncture treatment group, 6.8 ± 2.9 cm in the positive control (local anesthetics) group and 8.6 ± 3.3 cm in the negative control group (saline treatment). It seems that when the DFB was over 3.8 cm, the horse was lame; and when the DFB was between 0.5 and -2.4 cm, the horse was not lame. This indicates that the DFB could be used as an objective parameter to measure lameness in horses. But, the precise role of the DFB in lameness evaluation requires further clarification.

Like the lameness score, the acupuncture treatment decreased the DFB by 65% from 10.8 ± 2.4 cm to 3.8 ± 1.3 cm and only partially resolved lameness as compared with the local anesthetic bupivacaine which completely resolved lameness and the DFB returned to the baseline.

Acupuncture Mechanism

Acupuncture Effect on Neurotransmitters Including β-endorphin

In the pain threshold study, the results showed that the 2nd acupuncture treatment group induced an increase in plasma concentration of β-endorphin by 211.9% ($P<0.001$) at 0 time period and by 63.3% at 15', 60.3% at 30' and 43.3% at 60' time periods. All other acupuncture treatment groups induced a slight increase in plasma concentration of β-endorphin but without significance. As discussed above, the 2nd acupuncture treatment caused a stronger analgesic effect. This result coincides with the findings in which the stronger the analgesic

effect, the higher the β-endorphin content in the brain (Chen et al., 1982). Wu et al. (1995) also found that the release of β-endorphin increased significantly when the pain threshold increased significantly after EA treatment. There was a linear correlation between increases in β-endorphin and the body's ability to tolerate pain (Hamra et al., 1993). It seems that there is a significant correlation between the release of β-endorphin and the increase of pain threshold. The release of β-endorphin may be one of pathways in which acupuncture relieves pain in horses.

Studies show that forebrain region caudate nucleus (CN) is involved in acupuncture analgesia (He, 1987). A lesion in CN attenuated EA analgesia (EAA) in rabbits while CN stimulation enhanced the EAA (He and Xu, 1981). Abundant opioid peptides and opiate receptors are found in CN. Microinjection of naloxone into the CN temporarily blocked the EA analgesic effect (He and Xu, 1981). Xie et al. (1984) found that a positive correlation between the content of met-enkephalin in CN and the efficacy of EAA. Numerous studies suggest that other forebrain regions including septal area (SA), nucleus accumbens and nucleus amygdala may be also involved in acupuncture analgesia (He, 1987). In addition, Repeated EA stimulation significantly increased substance P, neurokin A and neuropeptide Y in the hippocampus and neuropeptide Y in the occipital cortex (Bucinskaite et al., 1994).

In order to probe into whether β-endorphin is involved in descending modulation of the somatosensory area (SII) of the cerebral cortex on the nucleus

centrum medianum (CM) of the thalamus and this mechanism of acupuncture analgesia, Dong et al. (1996) compared effects of electrical stimulation of SII and EA on β-endorphin content in the perfusate from the nucleus CM. They found that β-endorphin content in the perfusate was increased significantly by electrical stimulation of SII and not by EA, suggesting that the β-endorphin release from the nucleus CM was elevated by the stimulation of SII and was not influenced by EA. It indicates that β-endorphin may not be involved in the regulatory effect of EAA at the thalamus level (Dong et al., 1996). However, EA stimulation at the acupoint Zu-san-li induced an increase in the expression of c-fos and poropiomelanocortisn (POMC) in the hypothalamic arcuate nucleus (Arc) in rats (Zhang et al., 1996). An electrolytic lesion or surgical isolation of the Arc weakened the acupuncture analgesia (Quo et al., 1982). EA effect on the depressor-bradycardia was mediated by the β-endorphinergic projections from Arc and nucleus tractus solitarii (NTS) to the rostral ventrolateral medulla (Ku and Zou, 1993). Local application of naloxone into the preoptic area (PA) partially reversed EA analgesic effect in rabbits (Wu et al., 1984). These results suggest that Arc, NTS and PA may be involved in EAA.

Li et al. (1995) demonstrated that the release of β-endorphin and lue-enkephalin from the reticularis paragigantocellularis lateralis (RPGL) in the EA group was significantly higher than that in the control group ($P<0.05$). The RPGL may be the common relay station in the EAA and morphine analgesia (Li et al., 1995). EA treatment induced an increase in pain threshold and the level of leu-

enkephalin and β-endorphin release from PA, but a decrease in release of noradrenaline from PA (Wu et al., 1995). The high concentrations of β-endorphin are also found in the hypothalamus, PAG and locus ceruleus (Ferrante, 1993). Enkephalins including met- and leu-enkephalin are found in high concentrations in areas of the central nervous system: the periaquenductal gray (PAG), the rostroventral medulla, and laminae I, II, V, and X (Ferrante, 1993). The ventromedial medulla including the nuclei raphe magnus (RM) were found to be important in pain modulation as well (Beitz, 1992). Han et al. (1984) demonstrated a rich enkephalinergic innervation, but the absence of β-endorphin containing fibers in the spinal cord. β-endorphin might produce its analgesic effects by suppressing substance P (SP) release in the spinal cord, and met-enkephalin directly blocks release of SP, which may be related to what closed the gate in the spinal cord pain transmission system (Lumb and Jones, 1984). Using autoradiographic quantitative analysis of local cerebral metabolic rate of glucose, Jia et al. (1994) found the following central nervous structure most important to the EAA: T6-T8 and L1-L3 dorsal horns, locus coeruleus, nucleus raphe magnus, nucleus reticular gigantocellularis, PAG and hebeulae lateralis of thalanum. Using multimicropipettes for extracellular recording and iontophoresis, He and Dong (1983) found that morphine and etrophine produced a strong naloxone reversible inhibition on the spontaneous activity of the PAG neurones. EA induced a similar inhibitory effect on PAG neurones to iontophoretic morphine and etrophine, and the inhibition could be reversed by

iontophoretic naloxone. A correlation existed between the effects of EA and opiates ($P<0.0174$) (He and Dong, 1983). This result indicates that acupuncture signals may activate the central opioid peptidergic system to exert a control over the transmission of pain sensation in the PAG, to block the conveyance of nociceptive impulse at situ and at other relay stations through inhibitory systems.

Nucleus raphe magnus (NRM) of the ventromedial medulla and its descending inhibitory pathway have been proven to constitute a fundamental component in the central circuit responsible for the EA analgesic effect (He, 1987). A lesion in the medulla including the NRM resulted in a significant decrease in EA induced inhibition of viscero-somatic reflexes while stimulation of the NRM potentiated the EA inhibition (Du et al., 1978). Some evidences show that the serotoninergic descending pathway in the dorsolateral funiculi (DLF) transmits the inhibitory influence from the NRM to the spinal cord and mediates EAA. The 5-HT axons descending via the DLF project onto the dorsal horn where the distributions of the enkephalins and opiate receptors overlap and the nociceptive projecting neurons are localized (He, 1987). Modulation of pain transmission may result from the functional interaction between the endogenous opioid systems and 5-HT, or direct impingement of the 5-HT terminals on nociceptive neurons (Hoffert et al., 1983).

Another interesting study shows that EA induced an increase in the number of Fos-like immunoreactive (FLI) cell nuclei in the lumbar and sacral spinal cord of the rats exposed to noxious stimulus, while a decrease in the

number of the FLI in the locus coeruleus and the ventral part of PAG in the rats exposed to noxious stimulus. The noxious stimulus alone also increased significantly the number of FLI in the structure mentioned above while no obvious c-fos expression was shown in the control group (Wang et al., 1995). This result indicates that both EA and noxious stimulation may activate the intrinsic pain modulating system through the spinal dorsal horn, and then EA suppress the c-fos expression induced by noxious stimulation in the locus coeruleus and ventral part of the PAG.

Yonehara et al. (1992) found that tooth pulp stimulation evoked increase in release of substance P (SP) in the trigeminal nucleus caudalis (Vc-I,II), and this increase was inhibited by EA, or an SP antagonist. This result indicates that EAA may be through inhibition of noxious-evoked SP release from the afferent fibers and relay neurons and consequently block the ascending pain system.

EAA mediated by β-endorphin was also demonstrated in the cerebrospinal fluid (CSF) studies. Clement-Jones et al. (1980) found that EA effectively alleviated recurrent pain and significantly increased β-endorphin level in the lumbar CSF. EA induced an increase in ventricular CSF in patients with brain tumors, and there was a linear correlation between the percentage increase of β-endorphin and of pain threshold (Chen and Pan, 1984). EA also increased the pain threshold and CSF enkephalins in rabbits (Tsou et al., 1979) and monkeys (Huang et al., 1981). CSF is formed from the blood by secretory and filtration processes and is absorbed into the blood by way of subdural

venous sinuses and the spinal veins (Lumb and Jones, 1984). Because the skull forms an inelastic box and the brain-blood-CSF relationship is a reciprocating one, measurement of β-endorphin and enkephalins level in both CSF and peripheral blood may be a future potential approach to evaluate the involvement of neurotransmitters in the acupuncture analgesia.

The results of this study also show that pain threshold increased by 79% at during, 111% at 0' and by 85% at 15' time periods after acupuncture stimulation, while β-endorphin increased only at 0' time period after acupuncture treatment. It is possible that acupuncture induced the release of β-endorphin at first and then bound to the brain and other tissues quickly, thus the peripheral circulation of β-endorphin levels dropped. Based on the fact in that EA stimulation resulted in a decrease of plasma β-endorphin, Szczudlik and Lypka (1983) suggested that the increase of β-endorphin binding to the tissue receptor sites seem to be responsible for the peripheral (plasma) β-endorphin decrease after acupuncture. Panzer (1992) also noticed that EA affected the endogenous opioids in ways that modified gastrointestinal motility and yet did not change plasma (peripheral) β-endorphin levels. Our study result indicated that the release of β-endorphin from the central nervous system induced by EA into the peripheral circulation may be responsible for an increase in plasma level of β-endorphin at first, and then β-endorphin binding to the tissue receptors results in a drop of plasma β-endorphin, but this “drop” does not refer to a real decrease

in plasma β-endorphin but is relatively lower as compared with an increase in pain threshold.

Even though the 4th acupuncture treatment induced a significant increase in pain threshold, it did not change the plasma concentration of β-endorphin ($P>0.05$). This suggests that there may be other mechanisms by which acupuncture relieves pain. The naloxone (an opiate antagonist) did not inhibit EAA at high frequency (200 Hz) stimulation even though it completely reversed the EAA with low frequency (4 Hz), while parachlorophenylalanine (serotonin synthesis inhibitor) partially blocked high frequency (200 Hz) EAA but produced no effects on the low frequency (4 Hz) EAA (Cheng and Pomeranz, 1979). Another study showed intracerebroventricular saralasin (angiotension II antagonist) injection abolished or blocked EAA at 100 Hz stimulation but not at 15 Hz frequency, while 15 Hz EAA was blocked by intravenous injection of naloxone (Fedoseeva et al., 1990). Thus, the low frequency EAA may be mediated by endorphins while the high frequency EAA may be due to neuropeptides serotonin and angiotensin II. Serotonin enhancement of acupuncture analgesia was also demonstrated in other studies (Costa et al., 1982, Yu and Zhong, 1985 and Scherder and Bouma, 1993). EA induced a significant decrease in release of norepinephrine in preoptic area (Wu et al., 1995). In another study, acupuncture induced a significant increase in acetylcholine (Ach) with the elevation of pain threshold, while the dopaminergic system attenuated EAA (He and Xu, 1981 and Xu et al., 1983). Zhu et al. (1996)

found that orphnin FQ (OFQ), a newly discovered 17-amino-acid peptide, has a strong anti-EAA effect. EA stimulation with 100 Hz induced a significant increase in OFQ (Han, 1997).

In the hoof sole pressure study, β-endorphin tended to declined in the afternoon. In the control group (saline treatment), plasma concentration of β-endorphin at 0800 hours was 52.91± 17.8 pg/ml, decreased significantly to 31.51 ± 18.3 pg/ml at 1000 hours, and to 7.31 ± 2.9 pg/ml at 1330 hours. In the acupuncture treatment group, plasma concentration of β-endorphin decreased significantly to 38.76 ± 4.1 pg/ml at 0945 hours from 50.02 ± 2.8 pg/ml at 0800 hours, and acupuncture treatment (starting stimulation at 1015 hours and ending at 1100 hours) induced a significant increase in plasma concentration of β-endorphin to 63.44 ± 4.1 pg/ml at 1030 hours, 88.36 ± 2.1 pg/ml at 1045 hours, and 92.38 ± 3.6 pg/ml at 1100 hours. This increase in plasma level of β-endorphin induced by EA coincided with the pain threshold project. This diurnal variation in plasma β-endorphin was also noticed by other studies (Hamra et al., 1993 and Millington, 1986). Immunoreactive β-endorphin plasma concentrations were lower at 0600 hours, elevated at 0900 hours, dropped at 1200 hours and slightly elevated at 1500 hours, and then dropped again at 1800 hours and 2400 hours. The lowest level of β-endorphin were at 0600 hours and 1200 (Hamra et al., 1993). A diurnal rhythm of propiomelanocortin (POMC) mRNA was also found, and the variation of POMC mRNA paralleled that of β-endorphin (Millington, 1986).

Endogenous Hormones Including ACTH and Cortisol

In both pain threshold and the sole pressure projects, plasma levels of ACTH did not change among the time periods in all groups. This indicates that EA may not cause significant ACTH response. This result coincides with another study conducted in healthy volunteer people (Nappi et al., 1982). As β-endorphin is released in a one-to-one molar ratio with ACTH from the pituitary (Ferrante, 1993), why did not ACTH change while β-endorphin increased after EA stimulation? The change of ACTH should parallel with that of β-endorphin if both of them have the same origin (pituitary). The lack of significant change in plasma ACTH concentration after acupuncture stimulation in the present study suggests that β-endorphin may be released from other sources rather than the pituitary into peripheral blood. Some studies have demonstrated that EA stimulation induced the release of β-endorphin from the reticularis paragigantocellularis lateralis (RPGL) (Li et al., 1995), and from the preoptic area (PA) (Wu et al., 1995). The high concentrations of β-endorphin are also found in the hypothalamus, PAG and locus coeruleus (Ferrante, 1993).

The striking finding of the present study shows that EA induced a significant change in β-endorphin but not ACTH, which indicates that acupuncture induced-biochemical changes including the release of β-endorphin may not be simply due to an effect of "stress". However, Malizia et al. (1979) found that EA induced a significant increase in both β-endorphin and ACTH in healthy volunteer people. The different results may be related to variation of

species and subject statues. No other reports have been found about ACTH response to acupuncture stimulation.

In the pain threshold project, plasma concentration of cortisol did not change significantly (P>0.05) among different time periods in the 4th acupuncture treatment, positive control and negative control groups. However, plasma cortisol concentration decreased significantly by 20.4% to 33.1% at 30, 60, 90 120 and 180 minutes after treatment in AcuI group. For AcuII group, acupuncture treatment induced at first a significant 23% increase in plasma cortisol level at 0 time period, and then a significant decrease by 20.2% to 26.1 % 15 minutes after acupuncture stimulation. The 3rd acupuncture treatment (AcuIII) induced a significant decrease in plasma cortisol level at 60 up to 180 minutes after treatment (P<0.001). In the hoof sole pressure project, plasma cortisol concentration also decreased (P<0.001) 1 hour after EA stimulation. The reasons for the decrease of cortisol induced by acupuncture treatment remain to be determined. Diurnal variation of plasma cortisol concentration was found in mature Thoroughbred horses. The plasma cortisol level reached the peak at 0800 hours, dropped at 0900 hours, started to drop more from 1200 hours to 1800 hours, and dropped to the lowest level at 2400 hours (Nappi et al., 1982). In the hoof sole pressure project, this diurnal variation of plasma cortisol level was found in the control groups although the changes were not significant.

Acupuncture Pain Relief Model

Based on our present study and other reports, we propose that EAA may have the following pathways (figure 5-2):

1) Inhibition of pain transduction and transmission. More EA signals reach the dorsal horn of spinal cord, and reduces and/or inhibits painful signals to the spinal cord. EA may inhibit transduction and transmission of pain (figure 5-1 and 5-2). Inhibition of SP release by EA may be involved in this process.

2) Inhibition of pain ascending system. EA induced the release of β-endorphin which inhibits SP release, as a consequence, blocks onward transmission of the pain impulse to the high levels of the CNS (figure 5-1 and 5-2).

3) Activating the descending pain control system. The model of the descending inhibitory system is involved in 4 regions of the CNS (Raj, 1996) (figure 5-2):

a. Cortical and diencephalic system: The following nuclei and structure are involved in mediation of EAA: caudate nucleus (CN), septal area (SA), nucleus accumbens (NA), nucleus amygdala (Amy), occipital cortex (OC), hippocampus (HP), arcuate nucleus (Arc), habenular nucleus (HN), and preoptic area (PA).

b. PAG: PAG is the most important component of the descending pain control system.

c. Nucleus raphe magnus (NRM) in the ventromedial medulla: NRM receives excitatory input from PAG and sends the signals to serotonergic and noradrenergic fibers via the dorsolateral funiculus (DLF) in the medullary dorsal horn.

d. The spinal and medullary dorsal horn: They receive the terminal of axons from the NRM and adjacent nuclei. These descending fibers are serotonergic and terminate among nociceptive transmission cells in laminae I, IIo, and V, and thus selectively inhibit nociceptor neurons (Raj,1996). Norepinerphrine-containing neurons from the locus coeruleus are also involved in the descending pain control system.

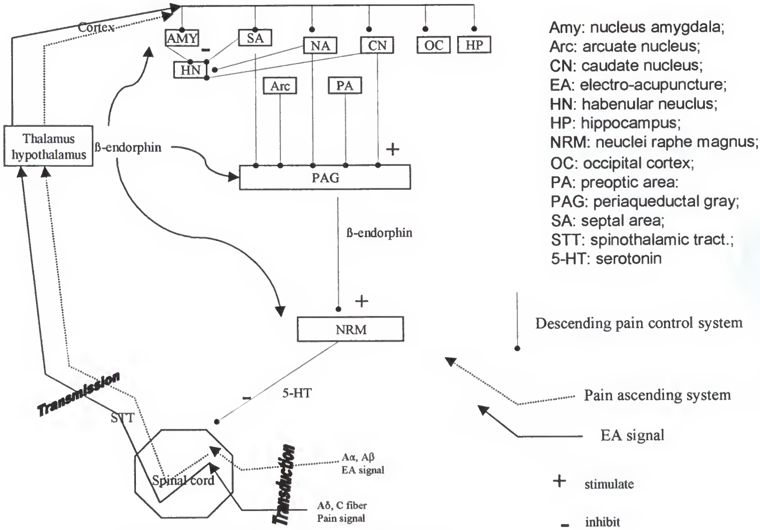


Figure 5-2 Acupuncture analgesia mechanism

CHAPTER 6
CONCLUSION

AcuII (4 local acupoints and 80-120 Hz) and AcuIV (2 local acupoints and 80-120 Hz) increased significantly ($P<0.01$) the pain threshold (HWRL) of the left (treated) limb in horses, while AcuI (2 local acupoints and 2 proximal acupoints, 80-120 Hz) and AcuIII (4 local acupoints and 20 Hz) did not alter the pain threshold in the treated limb. The results suggest that electro-acupuncture (EA) with high frequency (80-120 Hz) induce a stronger analgesic effect than that with low frequency (20 Hz). Low frequency EA induced a strong analgesia on the whole body, which is different from the local anesthetic. In clinical practice, high frequency stimulation of the acupoints close to the painful areas are recommended while low frequency stimulation of the acupoints far from the painful areas are recommended.

AcuII caused a stronger and longer analgesia than AcuIV, suggesting that acupuncture points potentiate each other. Four or more acupoints can be used for the treatment of painful conditions in the clinic.

EA can decrease the lameness score and reduce the degree of lameness significantly ($P<0.001$). This confirms that EA can be used for lame horses in the clinic. The afferent $A\alpha$ and $A\beta$ fibers seem to be more dominant in the

transduction of EA signals. The precise role of the afferents involved in electro-acupuncture analgesia (EAA) requires more studies.

The DFB increased significantly when the horse was lame. When the DFB was over 3.8 cm, the horse was lame; and when the DFB was between 0.5 and -2.4 cm, the horse was not lame. The DFB could be used as an objective parameter to measure lameness in horses. However, the precise role of the DFB in lameness evaluation requires further clarification.

EA significantly increased the pain threshold and and reduced the degree of lameness, and simultaneously increased plasma β-endorphin concentration. These results indicate that the release of β-endorphin may be one of the pathways in which acupuncture relieves the experimental pain. AcuIV (2 local acupoints, 80-120 Hz), however, induced an significant increase in PT but not β-endorphin, suggesting that some other pathways such as serotonin (5-HT) may also involve in mediation of EAA.

None of the acupuncture treatments altered the ACTH concentrations, which indicates that ACTH is not involved in EAA.

APPENDIX

Table A-1 % change in plasma concentration of ACTH in horses

Group	# of horse	% change in plasma concentration of ACTH (Mean ± s.e.)								P** value
		baseline*	0'	15'	30'	60'	90'	120'	180'	
AcuI	8	100.0±0 ^{a1}	101.9±15 $^{a}_{1}$	90.1±13 ^{a1}	92.1±8 ^{a1}	86.5±8 ^{a1}	87.2±11 ^{a1}	85.6±13 ^{a1}	98.8±19 ^{a1}	0.9521
AcuII	8	100.0±0 ^{a1}	82.9±3 ^{a1}	81.7±5 ^{a1}	74.5±5 ^{a1}	76.9±6 ^{a1}	86.4±9 ^{a1}	85.8±9 ^{a1}	81.3±7 ^{a1}	0.1610
AcuIII	4	100.0±0 ^{a1}	76.0±19 ^{a1}	72.5±18 ^{a1}	79.9 ±22 ^{a1}	87.0±25 ^{a1}	74.3±20 ^{a1}	77.6±19 ^{a1}	80.4±26 ^{a1}	0.9834
AcuIV	4	100.0±0 ^{a1}	101.6±16 ^{a1}	75.0±9 ^{a1}	89.4±14 ^{a1}	104.9±20 ^{a1}	118.2±24 ^{a1}	98.7±20 ^{a1}	97.0±16 ^{a1}	0.7719
PCG	5	100.0±0 ^{a1}	71.5±4 ^{a1}	71.0±10 ^{a1}	75.8±9 ^{a1}	59.1±4 ^{a1}	64.9±7 ^{a1}	61.6±6 ^{a1}	83.6±19 ^{a1}	0.0638
NCG	5	100.0±0 ^{a1}	94.1±5 ^{a1}	90.4±9 ^{a1}	93.6±16 ^{a1}	90.0±17 ^{a1}	88.0±19 ^{a1}	92.6±14 ^{a1}	101.4±23 ^{a1}	0.9977
P value *		0	0.3361	0.7191	0.6521	0.3032	0.3118	0.5503	0.9164	

* Baseline = (HWRL at -15' + HWRL at -5') ÷ 2;   % change in HWRL = (HWRL ÷ baseline) × 100.

**GLM analysis results. Mean values with different alphabetic superscripts are significant (P<0.05) within the group by the Duncan's Multiple Range Test.

***Mean values with different numerical superscripts are significantly different (P<0.01) between groups by the least squares means comparison.

Table A-2 % change in plasma concentration of cortisol in horses

Group	# of horse	% change in plasma concentration of cortisol (Mean ± s.e.)								P ** value
		baseline*	0'	15'	30'	60'	90'	120'	180'	
AcuI	8	$100.0\pm0^{d\,1}$	$92.8\pm6^{c,d\,1}$	$88.0\pm7^{b,c,d\,1}$	$79.6\pm5^{a,b,c\,1}$	$72.6\pm5^{a,b\,1}$	$66.9\pm6^{a\,1}$	$70.4\pm6^{a,b\,1}$	$79.3\pm8^{a,b,c\,1}$	0.0013
AcuII	8	$100.0\pm0^{b\,1}$	$123.0\pm7^{c\,1}$	$79.8\pm6^{a\,1}$	$79.1\pm5^{a\,1}$	$80.5\pm6^{a,b\,1}$	$73.9\pm10^{a\,1}$	$82.9\pm7^{a,b\,1}$	$79.0\pm6^{a\,1}$	0.0001
AcuIII	4	$100.0\pm0^{d\,1}$	$98.5\pm0^{d\,1}$	$92.0\pm4^{d\,1}$	$84.5\pm7^{b,c,d\,1}$	$71.0\pm11^{a,b,c\,1}$	$61.3\pm8^{a,b\,1}$	$51.8\pm7^{a\,1}$	$49.8\pm15^{a\,1}$	0.0002
AcuIV	4	$100.0\pm0^{a\,1}$	$102.3\pm14^{a\,1}$	$84.7\pm13^{a\,1}$	$86.3\pm12^{a\,1}$	$85.8\pm10^{a\,1}$	$95.8\pm23^{a\,1}$	$101.0\pm13^{a\,1}$	$93.0\pm3^{a\,1}$	0.9396
PCG	5	$100.0\pm0^{a\,1}$	$89.2\pm4^{a\,1}$	$107.6\pm15^{a\,1}$	$106.4\pm19^{a\,1}$	$89.8\pm11^{a\,1}$	$71.0\pm9^{a\,1}$	$66.0\pm13^{a\,1}$	$85.6\pm13^{a\,1}$	0.1697
NCG	5	$100.0\pm0^{a\,1}$	$100.6\pm7^{a\,1}$	$94.2\pm4^{a\,1}$	$91.0\pm3^{a\,1}$	$80.6\pm7^{a\,1}$	$74.8\pm11^{a\,1}$	$71.6\pm13^{a\,1}$	$82.8\pm9^{a\,1}$	0.0755
P value ***		0	0.0137	0.2974	0.3178	0.5271	0.5221	0.0662	0.1400	

* Baseline = (HWRL at -15' + HWRL at -5') ÷ 2;   % change in HWRL = (HWRL ÷ baseline) × 100.

**GLM analysis results. Mean values with different alphabetic superscripts are significant ($P<0.05$) within the group by the Duncan's Multiple Range Test.

***Mean values with different numerical superscripts are significantly different ($P<0.01$) between groups by the least squares means comparison.

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BIOGRAPHICAL SKETCH

Huisheng Xie was born in Tongnan, Sichuan province, PR China, on October 7, 1963. He lived in Tongnan, where he was educated from grade one all the way to high school. After receiving the Bachelor of Science degree of veterinary medicine from the Sichuan College of Animal Science and Veterinary Medicine (SCASVM) in August, 1983, he worked as assistant and staff veterinarian in the College of Veterinary Medicine, Beijing Agricultural University (BAU), PR China. From 1985 to 1988, he received in-service training for the master's degree in the area of veterinary acupuncture. After that, he was promoted to assistant professor at the same university. In 1993, he served as associate professor at the BAU until he moved to the USA.

In August of 1994, Huisheng enrolled at the University of Florida for his PhD training program. He is currently a member of Gamma Sigma Delta honor Society, the American Veterinary Medical Association, and the International Veterinary Acupuncture Society (IVAS). He is a regular IVAS herbal class faculty member. He was invited to speak on veterinary acupuncture in Ireland, Japan, Mexico, Colorado, Kentucky, Georgia, Florida, and California. Upon completion of his degree he plans to stay in Gainesville and look for an opportunity to take care of horses using the alternative approach.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edgar A. Ott, Chair
Professor of Animal Science

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Alistair I. Webb, CoChair
Professor of Veterinary Medicine

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Patrick T. Colahan
Associate Professor of
Veterinary Medicine

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Yann-Ching Hwang
Professor of Veterinary Anatomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edward L. Johnson
Associate professor of Animal Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1999

Dean, College of Agriculture

Dean, Graduate School